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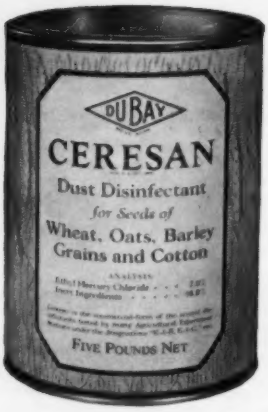

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PHYTOPATHOLOGY

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FUNGICIDAL EFFICIENCY OF CHEMICAL DUSTS CONTAINING FURFURAL DERIVATIVES

C. S. REDDY[1]

Uppal (9), in toxicity studies, showed that the furyl radical was no more toxic than an alkyl radical; and Flor (1), in preliminary studies, found that furfural in dilute solutions offered little promise as an effective fungicide. However, the latter author found that certain organic metallic salts derived from furfural showed high lethal action on fungous spores. Howe and Cation (3) showed that furfural had exceptional properties as a wood penetrant. Recent investigations (2, 6, 7, and 8) also have shown that certain organic compounds of mercury, in comparison with previous standard seed disinfectants, were mild in their action on the host, but efficient in the control of some of its seed-borne diseases.

These investigations led to a study of furfural as a source of organic radicals for metallic compounds, and the applicability of these compounds containing the furan ring as seed disinfectants for the control of corn diseases.

METHODS

Preliminary tests quickly showed that the most promising furfural materials were those containing mercury. Compounds of this type employed by the writer were mercury furoate, mercury furacrylate, mercury dithiofuroate, and others, made by definite procedures, of which the exact compositions were not determined. These toxic reagents were diluted with such fillers as asbestine, calcium carbonate, atomic sulphur, Kieselguhr, and talc.

The compositions of the fungicides studied are:

A1 —4 per cent mercury furacrylate + 96 per cent talc.
A2 —4 per cent mercury furacrylate + 25 per cent sodium furoate + 71 per cent talc.
A4 —8 per cent mercury furacrylate + 92 per cent talc.

[1] Research Fellow, Crop Protection Institute, 1927, on utilization of furfural products as seed disinfectants, stationed at the Plant Pathological Laboratories, under the direction of Dr. I. E. Melhus, Iowa State College, Ames, Iowa.

A6 —8 per cent mercury furacrylate + 25 per cent hydrofuramide + 67 per cent talc.
A'4 —24 per cent copper furacrylate + 76 per cent talc.
B1 —4 per cent mercury furoate + 96 per cent talc.
B4 —8 per cent mercury furoate + 25 per cent sodium furoate + 67 per cent talc.
B'2 —10 per cent copper furoate + 20 per cent sodium furoate + 70 per cent talc.
B'5 —10 per cent copper furoate + 5 per cent arsenous acid + 85 per cent talc.
D5 —5 per cent Paris green + 13 per cent furfural precipitated on 72 per cent talc by ammonia + 10 per cent sodium furoate.
E2C —10 per cent toxic reagent on talc made as follows: 100 cc. furfural were treated with 25 gm. sodium hydroxide and 90 cc. water. When the reaction was completed, 40 cc. ammonium hydroxide were added and the mixture was allowed to stand over night. Seven hundred and twenty gm. talc were suspended in 6 liters of water and the preceding mixture was added. With constant stirring, 80 gms. mercuric chloride dissolved in water were added and the talc and precipitate were allowed to settle. The supernatant liquid then was decanted. What remained was dried and pulverized.
E2C + —Same as E2C except that the mercuric chloride was increased to 100 gm.
E3 —5 per cent. toxic reagent (same as toxic reagent in E2C) + 10 per cent sodium furoate + 85 per cent talc.
G1 —Experimental dust G1 was made by uniting $HgCl_2$, hydrofuramide, and an inert filler such as talc. In the following experiments, it was made as follows: 10 grams hydrofuramide dissolved in acetone were added to 100 gms. talc and mixed. Twelve grams $HgCl_2$ dissolved in acetone were added, mixed, dried, and pulverized to such fineness as to adhere evenly to seed corn.
G3 —same as G1 but has, in addition, 10 per cent sodium furoate.
G7 —Half strength of G1 + 10 per cent sodium furoate.
G8 —One-half strength G3 + 5 per cent hydrofuramide.
67 —10 per cent mercuric chloride + 90 per cent of a mixture of furfural precipitated on Kieselguhr by ammonia.

Hereafter, these fungicides will be referred to by the designation preceding each fungicide, as shown above.

The first consideration in these studies was to obtain a large number of known compounds derived from furfural, especially those containing metals that were not too expensive to be used as seed-treatment materials. To this number were added similar compounds made by the writer. The latter compounds had certain advantages over the former in that they were made for a particular use, could be precipitated directly on the filler in the process of making, and were sometimes previously unknown compounds that were easily made.

In order to determine whether or not the radicals derived from furfural had special merit, they were compared with similar compounds having other organic radicals. The initial experiments were, for the most part, simple biological tests conducted by means of a constant-temperature germinator containing sand trays measuring 20 inches x 20 inches x 3 inches each. One-half inch of sand was spread on the bottom of each tray and covered with a musin cloth. The sand and cloth were made moderately wet and the corn was laid on the cloth in rows with the germ side up. Glass was placed on the top of each tray to retard loss of moisture and assure germination in a saturated atmosphere. Many materials were eliminated, because these simple tests showed that the development of *Diplodia zeae* (Schw.) Lev. on corn seed was not inhibited by them.

In the subsequent preliminary experiments, except for a few semifinal tests in soil in the greenhouse, extensive use was made of the visible-root sand-culture method (5). In these trials "nearly disease-free" seed and seed naturally infected with *Diplodia zeae* were used.

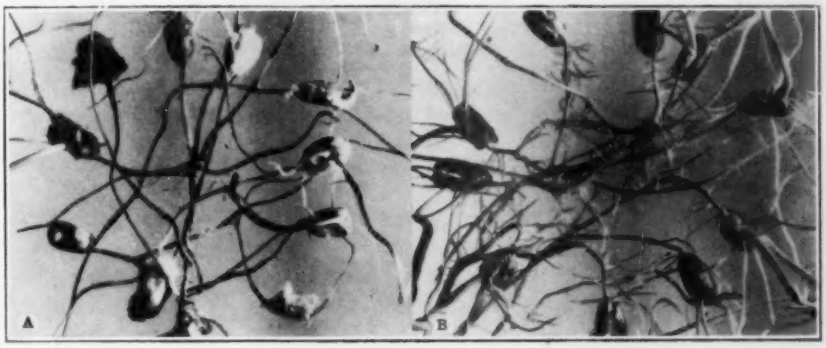

FIG. 1. Seedlings from the sand-tray germinator grown from Diplodia-infected seed corn. A, nontreated; B, treated with G1 dust fungicide.

The purposes of the preliminary experiments were to eliminate a large number of the less suitable disinfectants from expensive field trials and to determine approximately the proper strengths of the disinfectants that were to be tested further. In fact, careful manipulation of the preliminary biological tests seemed to be the most important phase in the development of this new seed disinfectant.

By using the "visible-root sand-culture method," conditions surrounding the germination and growth of the seedlings were largely controlled and it was possible at all times to note the correlation between the presence of disease or injury by the disinfectant and the amount of vegetative growth above the soil. (Fig. 1.) The final test to determine the most ef-

fective seed corn disinfectants was by comprehensive field experiments in which field stands and yields were recorded. Experiments were conducted at 15 separate places in Iowa and two in Illinois, so that the effects of the fungicides were measured under many conditions of corn growth. At Ames, Iowa, a typical experiment with each lot of seed consisted of ten replications of a 30-hill row planted with treated seed paired with 30-hill rows planted with nontreated seed. In each experiment, the number of fungicides was someaimes increased to nine without increasing the number of control rows. Experiments were conducted on several strains of corn, some of which were separated into lots predominantly infected with each of the dry-rot organisms, namely, *Diplodia zeae*, *Gibberella saubinetii* (Mont.) Sacc., and *Basisporium gallarum* M. Moll. Planting was by means

TABLE 1.—*Effect of a mixture of sodium furoate and furfuryl alcohol in different dilutions on the germination, presence of fungi, and the subsequent growth of the diseased seed corn. Ames, Iowa, 1927*

Ratio of mixture[a] to water	Approximate ratio of furfural to water	Percentage of germination	Percentage of kernels with fungi present	Condition of seedlings
Check	.....	90	70	normal
Check	.....	100	90	normal
Check	.....	90	90	normal
1:0	1:1	0	100	none
1:1	1:3	0	100	none
1:3	1:7	0	70	none
1:7	1:15	80	0	weak
1:15	1:31	100	0	very strong
1:31	1:63	100	0	normal
1:63	1:127	90	50	normal
1:127	1:255	100	50	normal
1:255	1:511	100	50	normal

[a] 100 cc. furfural, 100 cc. water, and 30 gm. sodium hydroxide.

of hand planters, and, to insure uniformity, the kernels were counted for each hill.

The importance of reducing the number of fungicides used in field experiments will be appreciated when it is stated that, even after eliminations, 16 acres were planted at Ames in 1927 and 22 acres in 1928.

EXPERIMENTAL RESULTS

Laboratory experiments. The mixture of compounds obtained from furfural by means of the Cannizzaro reaction (4) was used in different dilutions for treating Diplodia-infected seed. By this reaction, principally,

TABLE 2.—*Effect of experimental dust fungicides on Diplodia-infected seed corn as shown by percentage of germination and by percentage of diseased seedlings. Ames, Iowa, 1927*

Treatment	Percentage of germination after				Percentage of diseased seedlings after		
	3 Days	7 Days	8 Days	11 Days	7 Days	8 Days	11 Days
Check	0	90	90	90	10	30	40
A2a	20	70	70	70	10	10	20
A3	20	70	70	70	0	0	0
A4	60	80	80	80	0	0	0
A5	10	80	80	80	0	10	10
Check	0	80	80	80	30	40	80
A6	20	90	90	90	0	0	0
AC1	0	90	90	90	0	0	0
AB2	20	90	90	90	10	10	20
Check	0	70	70	70	30	30	50
ABC1	10	90	90	90	0	0	10
A1 + A'4	20	90	90	90	0	0	20
Check	20	70	70	70	20	40	40
B1	40	90	90	90	0	0	10
B2	60	90	90	90	0	10	20
B3	20	70	70	70	0	0	10
B'2 + B'5	40	80	80	80	0	0	30
Check	20	100	100	100	10	30	40
D5	40	90	90	90	10	20	20
57C	80	90	90	90	0	0	20
67	0	70	70	70	0	0	10
Check	40	70	70	70	10	20	60
E2	50	90	90	90	10	10	10
E2a	30	90	90	90	0	0	0
Check	40	90	90	90	0	20	20
E2b	20	90	90	90	0	0	0
E2c	50	90	90	90	0	10	30
E3	80	100	100	100	10	20	60
G1	10	100	100	100	0	0	10
G3	20	90	90	90	10	10	10
Check	20	90	90	90	10	10	20
G4	20	80	80	80	20	20	60
G5	40	70	70	70	20	30	50
G6	20	80	80	80	20	30	50
Check	20	100	100	100	10	30	50
G7	30	80	80	80	0	0	0
G8	30	100	100	100	10	10	20

sodium furoate and furfuryl alcohol are obtained. In the procedure 30 gm. sodium hydroxide, 100 cc. of water, and 100 cc. of furfural were used. The dilutions were made after the reaction was completed. The seed corn in units of ten kernels was soaked in the dilutions one and one-half hours, dried over night, and placed on the germinator (Table 1).

Table 1 shows that the solutions of the proper dilutions had a fungicidal effect on organisms associated with the germination of Diplodia-infected seed. When the solutions were too concentrated, they killed or injured the seed and allowed fungi to grow, when too dilute, fungi developed on the germinating kernels. It is of interest to note that fungi developed when the treatment was too strong or too weak, but did not develop within a rather wide range of intermediate strengths.

Table 2 gives the results of an experiment to determine the value of some of the experimental dust fungicides, using the sand-tray germinator. Diplodia-infected seed was used in units of ten kernels each.

In table 2 the data on the percentage of diseased seedlings indicate which dust disinfectants, under the conditions of this experiment, affect the development of seedling blight from Diplodia-infected seed. Likewise, the data on percentage of germination indicate which treatments, if any, may injure young corn plants. The dusts which did not retard germination or injure the germinating seed and at the same time control the disease were retained for further study (Fig. 2). On the basis of these data, certain other dusts may be retained and tried at either stronger or weaker strengths.

Table 3 and figure 3 present data on the effect of treating nearly-disease-free and Diplodia-infected seed corn with nine experimental dust fungicides. These data were obtained by the visible-root, sand-culture method in which five crystallization dishes, containing 15 kernels each, were used as a unit. The controls consisted of two control units of untreated seed in each instance. The series in which nearly-disease-free seed was used and one series in which Diplodia-infected seed was used were exposed to medium temperature conditions (16° to 20° C.). A diseased-seed series was placed under high-temperature conditions (22° to 30° C.) and a similar series under low-temperature conditions (10° to 15° C.).

The data in table 3 and figure 3, B, C, and D, show that treated Diplodia-infected seed corn usually emerged faster than nontreated seed of the same kind.

Figure 3, B, C, and D, shows that, excepting No. 3, all the dusts employed in these experiments increased the number of emerging plants. Number 3 was especially injurious at the low-temperature range.

The data in figure 3, E, indicate that none of the dusts was injurious to good seed corn at the medium-temperature range and that No. 2 was

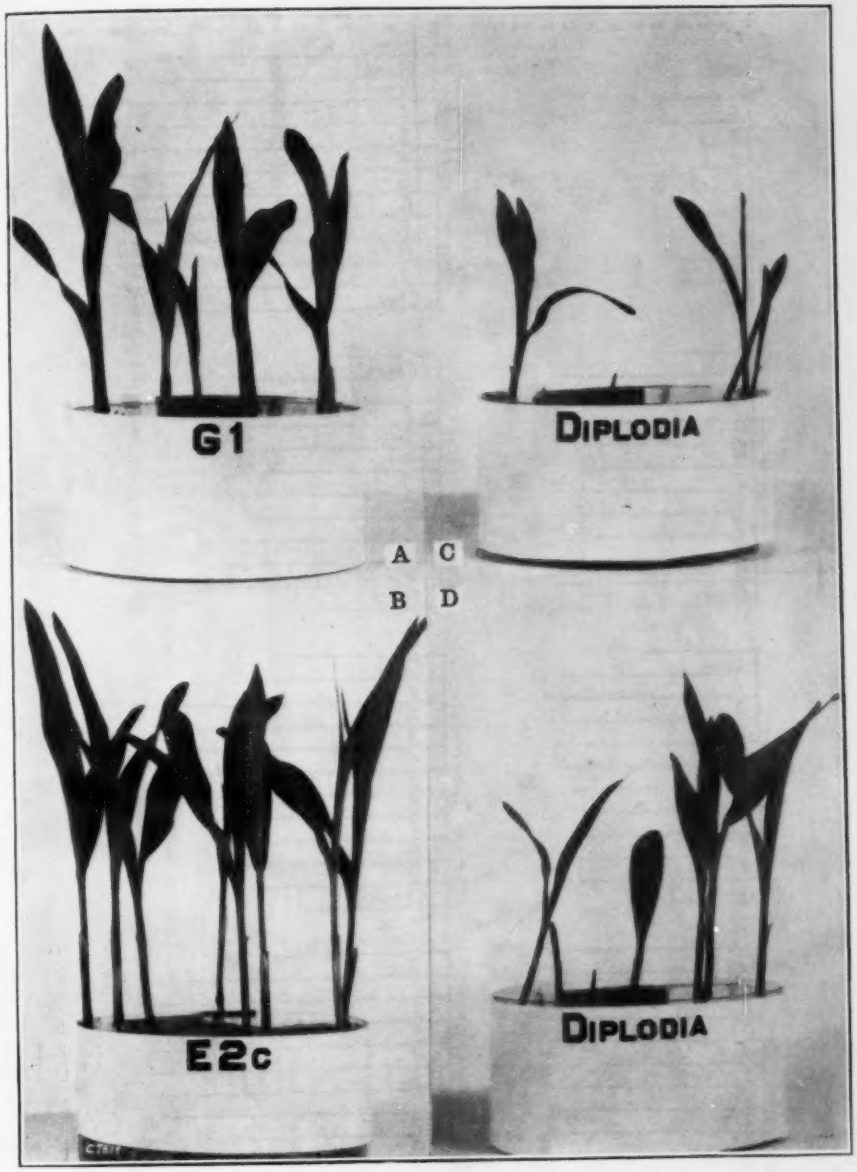

FIG. 2. Seedlings from Diplodia-infected seed corn. A, treated with G1; B, treated with E2C; C, D, nontreated.

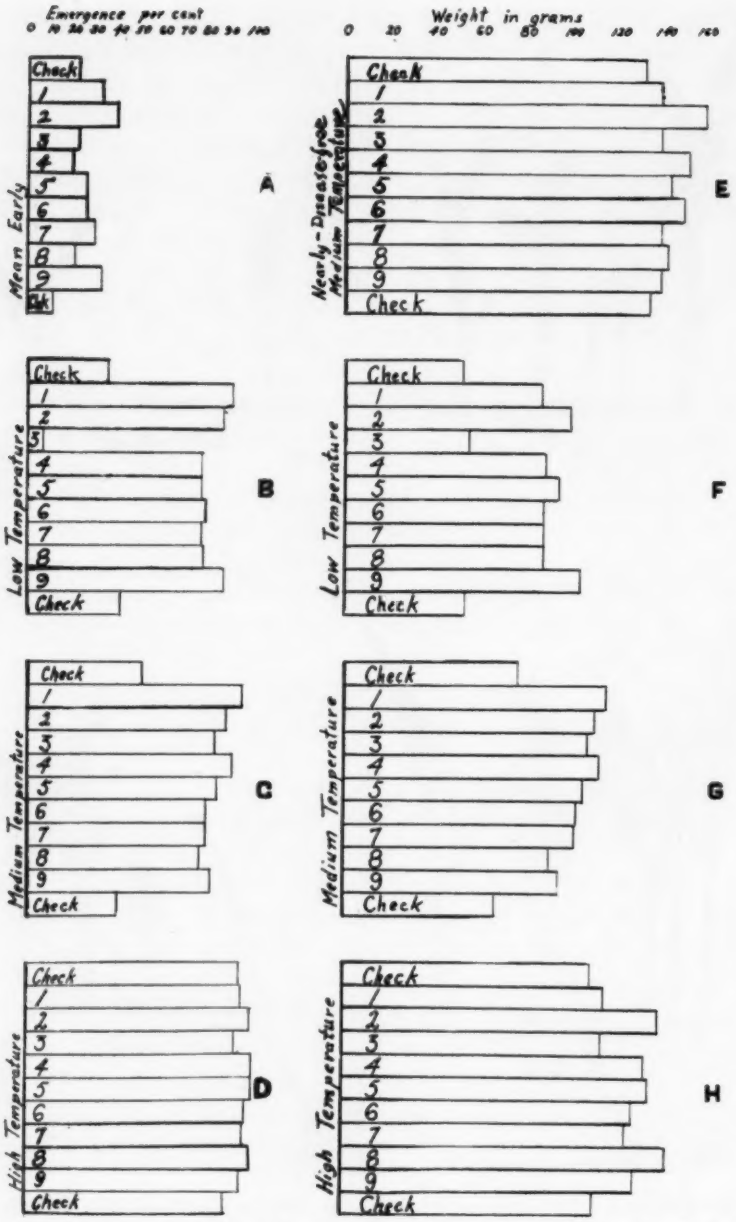

FIG. 3, A. The means of the early-emergence data given in table 3. B, C, D. Graphs showing seedling emergence, under temperature conditions, from Diplodia-infected seed corn, nontreated and treated with nine trial dusts, respectively, table 3. E, F, G, H. Graphs showing the green weight of plants from nearly-disease-free seed corn, nontreated and treated with nine trial dusts and grown under different temperature conditions, table 3.

TABLE 3.—*Data on germination and early growth obtained by the visible-root sand-culture method, to indicate the values of a number of trial dust fungicides for treatment of nearly-disease-free and Diplodia-infected seed corn*

Treatment No.	Nearly-disease-free seed				Diplodia-infected seed								
	Emergence		Green wt. in gms.		Emergence percentage at first and last reading						Green wt. in grams		
					High temperature		Medium temperature		Low temperature				
	No.	Per cent	Total	Corrected to perfect stand	First	Final	First	Final	First	Final	High temp.	Med. temp.	Low temp.	Av. wt.
0	61	81	107	131.6	32	94	32	51	2	36	110	77	52	79.7
1	74	99	137	138.9	37	95	61	95	0	91	116	116	87	106.3
2	69	92	146	158.7	71	99	32	88	16	87	140	111	100	117.0
3	73	97	135	138.7	16	92	52	83	0	7	115	108	55	92.7
4	64	85	129	151.2	16	100	40	91	5	77	134	113	89	112.0
5	72	96	137	142.7	27	100	51	84	3	77	136	106	95	112.3
6	66	88	131	148.9	33	97	39	79	8	79	128	103	88	106.3
7	69	92	128	139.2	25	96	64	79	1	76	126	102	88	105.3
8	71	95	134	141.5	1	99	48	76	15	77	144	91	88	107.7
9	69	92	128	139.1	51	95	31	81	17	87	130	95	104	109.7
0	66	88	118	134.1	12	88	16	40	4	41	112	67	53	77.3

TABLE 4.—*Effect of seed treatments of corn as determined by four replications of 20 hills each of Diplodia-infected seed and two replications of 20 hills each of nearly-disease-free seed. Ames, Iowa, 1927*

Dust disinfectant	Field stand Diplodia-infected		Percentage of increase		Field stand Nearly-disease-free		Percentage of increase or decrease	
	Non-treated	Treated	Stand	Yield	Non-treated	Treated	Stand	Yield
	Number	Number			Number	Number		
A2	71.5	110	53.9	65.3	74	77	4.1	1.4
A6	71.5	104	45.5	45.2	74	74	0	8.8
Ac1	74.5	111	49.0	32.4	75	75	0	− 6.2
ABC1	74.5	98	31.6	36.8	75	77	2.7	9.5
B1	79	111	39.3	45.2	76	74	− 2.6	− 13.0
D5	79	105	32.9	16.2	76	75	− 1.3	− 1.0
67	83.5	124	48.5	43.5	77	71	− 7.8	10.6
E3	83.5	105	25.8	33.0	77	72	− 6.5	0
E2C	84	109	29.8	30.8	75	74	− 1.3	5.0
G3	84	108	28.6	25.9	75	73	− 2.7	− 1.6
G8	83	103	24.1	21.2	73.5	71	− 3.4	− 6.6
Can. 1: 32	83	95	14.5	16.7	73.5	68	− 7.5	− 11.2
A1 + A'4	88.5	107	20.9	10.9	73	70	− 4.1	3.5
E2C + M2 per cent	88.5	115	30.0	11.5	73	72	− 1.4	− 7.2
G1 + E2C	75	97	29.3	33.5	68	70	2.9	1.2
Mercurochrome (1 per cent in talc)	59[a]	33	− 44.1	− 34.2	35[b]	33	− 5.7	− 12.1

[a] Two replications.

[b] One replication.

outstandingly beneficial. It is of interest to note that, even on nearly-disease-free seed, the injurious character of dust No. 3 was not brought out at this temperature range, a range within its narrow limits of safety. This emphasizes the necessity of trials at different temperatures.

Figure 3, F, G, and H, shows that all the dusts except No. 3 were markedly beneficial to diseased seed. There were indications of benefit from No. 3 only within the medium-temperature range and, therefore, it should be discarded because of its narrow limits of safety. Dust No. 2 was consistently effective at all temperatures and in this experiment was the outstanding dust fungicide. Number 8 was especially satisfactory at high temperatures, while No. 9 was better than No. 8 at low temperatures. Dusts 4, 5, 6, and 7 were uniformly effective, but never the best within any one temperature range.

The results of a preliminary field experiment (Table 4) show that many dust fungicides increase the yield from diseased seed corn, but that not all of them are safe in general practice because of their effect on nearly-disease-free seed.

Tables 5 and 6 show the results of experiments performed by Dr. J. R. Holbert, at Bloomington, Illinois, in which he used one of the furfural-mercury dust fungicides, E2C. The data on the use of a commercial dust are included for purposes of comparison.

The data in tables 5 and 6 show that E2C Dust is a fungicide which increases yields from diseased seed corn, comparable to results from using a commercial dust, and is especially satisfactory from the standpoint of its effect on nearly-disease-free seed.

Table 7 shows the performance of six "furfural" fungicides and one commercial dust fungicide in field experiments at Ames in 1927, on three strains of corn from which a number of nearly-disease-free and diseased lots of seed had been separated.

E2C + gave good results on Diplodia-infected and Basisporium-infected seed, but caused injury to Funk's 176A strain of yellow dent seed corn. This indicates that the modification of E2C, designated E2C +, was not advisable because E2C did not injure the same strain of corn (Table 5) in experiments in Illinois. The fungicide G7, which was approximately half the strength of G1, gave the highest increases in acre yield in three out of five experiments in which it was used with six other disinfectants. This was one of the strong indications that G1 contained more than enough toxic reagent for effective control of the dry-rot seedling blights of corn and that this toxic reagent was effective and noninjurious over a wide range of concentration. Mercury furacrylate, as in A4, gave higher increases in acre yields than the commercial dust in four of the five experiments in which it was used.

TABLE 5.—*Field stands and acre yields of yellow dent corn nontreated and treated with Bayer dust and a furfural-mercury dust (E2C).*[a] *Bloomington, Illinois, 1927*

Kind of seed	Treatment	No. of replications	Field stand		Acre yield		Increase or decrease	Odds
			Non-treated	Treated	Non-treated	Treated	Bu. per acre	
Nearly-disease-free	BD	8	91.9	95.3	46.3	48.4	2.1	7:1
"	E2C	8	92.2	93.2	48.6	49.3	0.7	2:1
"	BD	8	87.0	84.4	56.2	53.5	− 2.7	14:1
"	E2C	8	93.5	93.2	56.5	56.2	− 0.3	9:1
"	BD						− 0.1	1:1
"	E2C						1.7	29:1
"	BD						− 0.1	1:1
"	E2C						− 1.2	2:1
"	BD						3.5	5:1
"	E2C						1.5	13:1
"	BD						− 2.0	1:1
"	E2C						1.5	3:1
"	BD						1.0	1:1
"	E2C						2.5	14:1
"	BD						2.0	3:1
"	E2C						3.7	29:1
Commercial	BD	8	87.5	88.5	50.0	51.1	1.1	2:1
"	E2C	8	86.7	84.4	50.4	50.4		
"	BD	8	80.5	80.2	40.6	44.0	3.4	8:1
"	E2C	8	83.9	84.9	42.9	46.2	3.5	151:1
Diplodia-infected	BD	8	56.5	81.3	32.0	47.9	15.9	506:1
"	E2C	8	56.8	85.9	34.5	51.9	17.4	9999:1
"	BD						11.9	232:1
"	E2C						13.7	35:1
"	BD						12.3	175:1
"	E2C						12.9	61:1
"	BD						15.7	964:1
"	E2C						6.1	21:1

TABLE 5.—(*Continued*)

Kind of seed	Treatment	No. of replications	Field stand		Acre yield		Increase or decrease	Odds
			Non-treated	Treated	Non-treated	Treated	Bu. per acre	
Gibberella-infected	BD	8	71.1	90.1	39.2	53.9	14.7	270:1
"	E2C	8	68.2	87.5	37.3	53.1	15.6	898:1
"	BD						4.6	26:1
"	E2C						2.3	20:1
Fusarium-infected	BD	8	89.0	92.2	49.4	55.0	5.6	132:1
"	E2C	8	87.2	88.0	48.6	52.5	3.9	8:1
Cephalosporium-infected	BD	8	90.6	89.6	51.1	51.1		
"	E2C	8	87.0	84.4	51.3	49.9	− 1.4	4:1
Basisporium-infected	BD	8	76.3	90.6	39.0	44.2	5.2	11:1
"	E2C	8	83.3	92.2	43.3	45.6	2.3	5:1
"	BD						4.3	94:1
"	E2C						2.7	27:1
"	BD						1.4	2:1
"	E2C						6.2	165:1

[a] Toxic reagent is furfural, after undergoing Cannizzaro reaction, and mercuric chloride.

TABLE 6. *Summary of increases and decreases in acre yields following seed treatment with Bayer dust and a furfural-mercury dust (E2C). Bloomington, Illinois, 1927*

Kinds of seed	Fungicide	No. experiments	Av. increase or decrease in bushels per A. for all experiments
Nearly-disease-free seed	Bayer Dust	8	0.46
	E2C		1.26
Commercial seed	Bayer Dust	2	2.25
	E2C		1.75
Diplodia-infected seed	Bayer Dust	4	13.95
	E2C		12.52
Gibberella-infected seed	Bayer Dust	2	9.65
	E2C		8.95
Fusarium-infected seed	Bayer Dust	1	5.6
	E2C		3.9
Cephalosporium-infected seed	Bayer Dust	1	0
	E2C		−1.4
Basisporium-infected seed	Bayer Dust	3	3.97
	E2C		3.73

$B^{12} + B^{15}$ was the only organic copper compound used in the experiments reported in table 7. This compound was ineffective on Gibberella-infected seed. On Diplodia-infected seed it gave about half as much increase as the commercial dust in the same experiments. G1 had ranked high in the preliminary experiments and was used in all the field experiments reported in table 7. The results showed that it was better than the commercial dust in six of the eight field experiments, and the results obtained from the use of G7 showed that G1 might be made more satisfactory by decreasing the percentage of toxic reagent in the dust fungicide.

The effectiveness of two of the furfural-mercury dusts, shown in table 7, is shown more in detail in table 8 and the results are presented graphically in figure 4.

Table 8 and figure 4 show that G1 dust is a promising seed-corn fungicide because it does not injure good seed and materially increases the yields from diseased seed. Major emphasis on perfecting this compound was given in the subsequent year's investigations. Marked improvement has been made, especially in modifying the strength of the toxic reagent. Also, other solvents have been found that can be used in uniting the hydrofuramide and mercury.

Tables 9 and 10 show the effects on field stand and yield resulting from the seed treatment of two different lots of seed corn with a number of dust fungicides.

TABLE 7.—*Field stands and acre yields from yellow dent seed corn nontreated and treated with dust fungicide. Ames, Iowa, 1927*

Kind of corn	Seed treatment	Field stand	Increase in stand following treatment	Mean acre yield	Increase or decrease in acre yield following treatment		Odds
		Per cent	Per cent	Bu.	Bu.	Per cent	
Diplodia-infected U. S. D. A. cross 250 seed	Check	61.5		29.2			
	BD	80.5	30.9	35.3	6.1	20.9	262:1
	E2C+	70.5	14.6	31.7	2.5	8.6	13:1
	G1	79.2	28.7	38.5	9.3	31.8	9999:1
	B4	57.4	−6.8	28.1	−1.1	−3.8	3:1
	G7	77.0	25.2	39.3	10.1	34.6	9999:1
	B'2+B'5	65.0	7.0	32.3	3.1	10.6	23:1
	A4	73.0	18.7	35.5	6.3	21.6	1428:1
Nearly-disease-free U. S. D. A. cross 250 seed	Check	91.3		36.3			
	BD	91.8	0.5	35.6	−0.7	−1.9	3:1
	E2C+	89.3	−2.2	36.0	−0.3	−0.8	1:1
	G1	92.2	0.9	36.4	0.1	0.3	1:1
	B4	82.7	−9.5	35.1	−1.2	−3.3	4:1
	G7	90.7	−0.7	37.6	1.3	3.6	5:1
	B'2+B'5	94.0	2.9	37.1	0.8	2.2	3:1
	A4	90.0	−1.5	37.6	1.3	3.6	5:1
Gibberella-infected Funk's 176A seed	Check	79.8		36.9			
	BD	84.2	5.4	38.2	1.3	3.5	7:1
	E2C+	83.5	4.6	36.8	−0.1	−0.3	1:1
	G1	84.7	7.3	41.1	4.2	11.4	402:1
	B4	80.2	0.4	33.1	−3.8	−10.3	1666:1
	G7	82.8	3.8	39.9	3.0	8.1	16:1
	B'2+B'5	78.3	−1.9	35.6	−1.3	−3.5	6:1
	A4	83.1	4.6	40.9	10.8	4.0	416:1
Nearly-disease-free Funk's 176A seed	Check	94.9		42.2			
	BD	95.3	0.5	40.7	−1.5	−3.6	2:1
	E2C+	93.0	−1.9	36.9	−5.3	−12.6	132:1
	G1	95.5	0.7	41.4	−0.8	−1.9	2:1
	B4	93.7	−1.2	39.7	−2.5	−5.9	6:1
	G7	94.5	−0.4	40.1	−2.1	−5.0	4:1
	B'2+B'5	95.0	0.2	40.4	−1.8	−4.3	3:1
	A4	95.5	0.7	40.2	−2.0	−4.7	9:1
Diplodia-infected Funk's 176A seed	Check	57.0		25.5			
	BD	84.8	48.8	36.5	11.0	43.2	9999:1
	E2C+	84.7	48.6	36.2	10.7	42.0	9999:1
	G1	84.8	48.8	40.7	15.2	59.6	9999:1
	B4	58.2	2.0	25.6	0.1	0.4	1:1
	G7	84.5	48.3	41.6	16.1	63.2	9999:1
	B'2+B'5	70.5	23.7	31.6	6.1	23.9	9999:1
	A4	79.5	39.5	40.5	15.0	58.8	9999:1
Diplodia-infected Murphy strain seed	Check	59.7		29.0			
	BD	75.3	26.3	37.0	8.0	27.6	2399:1
	Sem. Jr.	78.0	30.7	37.3	8.3	28.6	9999:1
	G1	71.0	19.0	34.6	5.6	19.3	276:1
	E2C+	71.5	19.8	36.2	7.2	24.8	1999:1
Basisporium-infected Murphy strain seed	Check	64.7		26.5			
	BD	77.5	19.8	31.7	5.2	19.6	9999:1
	Sem. Jr.	78.6	21.4	30.6	4.1	16.1	1666:1
	G1	75.4	16.5	28.8	2.3	9.0	8:1
	E2C+	78.4	21.2	31.1	4.6	18.0	73:1
Nearly-disease-free Murphy strain seed	Check	91.4		32.1			
	BD	94.9	3.8	33.2	1.1	3.1	7:1
	Sem. Jr.	92.7	1.4	31.9	−0.2	−0.6	1:1
	G1	93.2	2.0	33.3	1.2	3.7	3:1
	E2C+	93.5	2.3	35.0	2.9	9.3	11:1

TABLE 8.—*Yields from nearly-disease-free and diseased seed corn untreated and treated with Bayer Dust and two dust disinfectants made from furfural and mercury. Iowa Agricultural Experiment Station, Ames, Iowa, 1927*

Kind of corn	Dust disinfectant	No. of replications	Acre yields		Increase or decrease		Odds
			Untreated Bu.	Treated Bu.	Bu.	P. ct.	
Disease-free (250)	Bayer Dust	10	36.3	35.6	−0.7	−1.9	3:1
" (Mur)	"	10	32.1	33.2	1.1	3.1	7:1
" (176A)	"	10	42.2	40.7	−1.5	−3.6	2:1
Mean		30	36.9	36.5	−0.4	−0.8	
Disease-free (250)	G1	10	36.3	36.4	0.1	0.3	1:1
" (Mur)	"	10	32.1	33.3	1.2	3.7	3:1
" (176A)	"	10	42.2	41.4	−0.8	1.9	2:1
Mean		30	36.9	37.0	0.1	0.3	
Disease-free (250)	E2C+	10	36.3	36.0	−0.3	−0.8	1:1
" (Mur)	"	10	32.1	35.0	2.9	9.3	11:1
" (176A)	"	10	42.2	36.9	−5.3	−12.6	132:1
Mean		30	36.9	36.0	−0.9	−2.4	
Diplodia-infected (250)	Bayer Dust	10	29.2	35.3	6.1	20.9	262:1
" (Mur)	"	10	29.0	37.0	8.0	27.6	2399:1
" (176A)	"	10	25.5	36.5	11.0	43.2	9999:1
Mean		30	27.9	36.3	8.4	30.1	
Diplodia-infected (250)	G1	10	29.2	38.5	9.3	31.8	9999:1
" (Mur)	"	10	29.0	34.6	5.6	19.3	276:1
" (176A)	"	10	25.5	40.7	15.2	59.6	9999:1
Mean		30	27.9	37.9	10.0	35.9	
Diplodia-infected (250)	E2C+	10	29.2	31.7	2.5	8.6	13:1
" (Mur)	"	10	29.0	36.2	7.2	24.8	1999:1
" (176A)	"	10	25.5	36.2	10.7	42.0	9999:1
Mean		30	27.9	34.7	6.8	24.4	

Tables 9 and 10 show that Bayer Dust used on these two lots of corn ranked first in its effect on yields, although it did not increase the field stand so much as some of the experimental dust fungicides.

Table 11 shows the effect of one of the experimental dust disinfectants on four lots of seed corn, two of which were hybrid seed.

The data in table 11 show that the crossed corn used was not benefited by seed treatment with E2C+, while an open pollinated variety in the same

FIG. 4.—Yields from good seed corn and diseased seed corn nontreated and treated with Bayer Dust and two dust disinfectants made from furfural and mercury.

TABLE 9.—*Percentage increases in field stands and acre yields from one lot of poor seed corn following treatment with certain chemical dusts. Ames, Iowa, 1927*

Rank	Dust disinfectants	Percentage increase in		Odds
		Stand	Yield	
1	Bayer Dust	12.3	23.7	9999:1
2	E2C	12.4	15.0	4999:1
3	E2C+	7.5	13.6	207:1
4	G7	4.5	12.7	147:1
5	E2C+G1	13.2	11.9	158:1
6	Sem. Jr.	8.2	11.9	42:1
7	K4	7.5	10.0	33:1
8	Old Homestead	1.7	8.5	11:1
9	E2C5	9.3	7.2	11:1
10	MC1	3.0	5.7	7:1
11	G1	9.6	4.4	9:1
12	$B^{12}+B^{15}$	2.6	4.0	3:1
13	A4	2.9	0.3	1:1

TABLE 10.—*Percentage increase or decrease in field stand and acre yield from one lot of poor seed corn treated with certain chemical dusts. Ames, Iowa, 1927*

Rank	Dust disinfectants	Percentage increase or decrease in		Odds
		Field stand	Yield	
1	Bayer Dust	8.0	+15.4	140:1
2	E2C+G1	12.4	+14.2	50:1
3	Sem. Jr.	11.2	+13.1	119:1
4	E2C (half strength)	8.5	+10.9	2249:1
5	MC1 per cent	0.8	+ 8.5	14:1
6	E2C+	12.7	+ 8.2	49:1
7	G1	16.6	+ 3.5	6:1
8	G7	5.1	+ 3.4	10:1
9	E2C	11.8	+ 3.3	4:1
10	$B^{12}+B^{15}$	− 2.4	− 0.6	1:1
11	HSD	− 4.4	− 0.9	1:1
12	K4	0.0	− 6.5	9:1
13	A4	0.6	− 4.7	8:1

experimental field was materially benefited by the same seed treatment. Other evidence also indicates that some varieties of corn are benefited more than others by the same seed treatments.

Past experience has shown that seed treatments of Cephalosporium-infected seed (black-bundle disease) have not consistently given beneficial results. As a rule, this disease does not affect germination or early vigor

TABLE 11.—*Effect of E2C+ on four lots of seed corn planted at Ames, Iowa, 1927*

Kind of corn	No. replications	Acre yield		Increase or decrease		Odds
		Nontreated	Treated	Bu.	P. ct.	
Nearly-disease-free	10	38.4	42.0	3.6	9.4	37:2
Diplodia-infected	10	34.9	40.3	5.4	15.5	9999:1
Crossed corn A	21	39.3	37.5	−1.8	−4.6	11:1
Crossed corn B	21	38.3	38.7	0.4	1.0	2:1

of the seedlings. Table 12 shows the results of a seed-treatment experiment in which Cephalosporium-infected seed was used.

TABLE 12.—*Effect of dust disinfectant E2C5 on stand and yield of nearly-disease-free and Cephalosporium-infected Funk's Yellow Dent seed corn. Ames, Iowa, 1927*

Kind of seed	No. of replications	Stand		Increase		Acre yield		Increase		Odds
		Treated	Non-treated	No. plants	Per cent	Treated	Non-treated	Bu.	P. et.	
N. D. F.	10	278	266	12	4.5	19.0	19.3	0.3	1.6	2:1
Cephalosporium-infected	10	224	190	34	17.9	23.4	19.4	4.0	20.6	484:1

In this experiment, the treatment has been of decided benefit to Cephalosporium-infected seed, increasing the field stand 17.9 per cent and the yield four bushels per acre, or 20.6 per cent.

Experiments on sweet-corn-seed treatment were conducted at two places in Iowa in 1927. The experimental method was similar to that used with dent corn at different places in the State. The seed was the same as that used for general planting by the canning company cooperating in the experiment.

The sweet-corn tests at Story City were replicated four times for each treatment. The variety grown in these tests was Golden Bantam. Scarcely any rain fell from the time of planting to harvest time. Moisture was outstandingly the limiting factor for yield and for this reason it was an unfavorable year to determine the value of seed-treatment materials.

The stand and yield data are presented in table 13.

In corn-seed treatment experiments there is usually a high correlation between increases in field stand and in yield. Table 13 shows that, in this experiment, the largest increase in field stands were not followed by the

TABLE 13.—*Data on field stands and acre yields of Golden Bantam sweet corn, prime for canning, from seed-treatment experiments at Story City, Iowa, 1927*

Dust disinfectants	Field stands		Acre yields		Increase or decrease	
	Non-treated No.	Treated No.	Non-treated Lbs.	Treated Lbs.	Stand P. ct.	Yield P. ct.
Sem. Jr.	998	1037	4938	5667	3.9	14.8
Sem. filler	998	980	4938	5208	− 1.8	5.5
G1	998	1152	4700	4842	15.4	3.0
Talc	1049	1106	4700	4958	5.4	5.5
E2C+	965	1032	4700	4942	6.9	5.2
E2C	965	986	4350	4422	2.2	1.7
Bayer Dust	938	957	4350	4210	2.0	−3.2
E2C+G1	911	1026	4350	4250	12.6	−2.3

largest increases in yield. These unusual results are probably due to drought during this season.

The sweet-corn plots at Grimes, Iowa, were not harvested; hence only field-stand data are presented in table 14.

TABLE 14.—*Effect of dust seed treatments on field stands of narrow-grain sweet corn (var. Evergreen) planted at Grimes, Iowa, 1927*

Dust disinfectants	Average of two checks		Increase or decrease
	Nontreated	Treated	
	No.	No.	P. ct.
Sem. Jr.	742	755	1.8
Sem. filler	742	656	−11.6
G1	742	812	9.4
Talc	742	730	− 1.6
E2C+	742	799	7.7
BD	677.5	676	− 0.2
E2C+G1	672	750	11.6

In this experiment the combination of E2C and G1 proved better than either one alone or any of the other disinfectants used.

Experiments were conducted in districts 2, 5, 7, and 10 in connection with the Iowa State yield tests to determine the seed-disinfection value of Bayer Dust, G1, and E2C+. In each of the four districts where these test fields were located, a composite was made with seed obtained from 12 separate farmers to represent average seed not specially selected. From each of the four composite lots four plantings were made, the first of which was

treated with Bayer Dust, the second with G1, and the fourth with E2C+. The third planting was of nontreated seed. In each row was planted the same number of seeds from each of the 12 separate farmers.

Each of four separate lots of seed, entered regularly in the corn yield test by the several growers, was divided into four portions and given the same treatments as the composite lots. These were planted in the same four districts as the composite lots and in similar manner.

The results obtained are shown in table 15 where the acre yields in bushels are given for each trial dust and for the nontreated, or check seed, in each district, together with the average yield for all the districts. No significant differences in yield between treated and nontreated composite lots of seed were obtained. Seed treatments did result in increased yields, however, when used with the four regular entries in the yield test; increases ranging from 0.74 to 2.08 bushels were obtained, each of the treated samples yielding more than the nontreated lots.

TABLE 15.—*Acre yields in bushels obtained from nonselected and specially selected seed nontreated and treated with two furfural and one commercial dust*

Kind of treatment	Acre yields in bushels				
	Dist. 2	Dist. 5	Dist. 7	Dist. 10	Average
Composite seed:					
Bayer Dust	33.59	44.03	50.86	46.74	43.81
G1	31.43	47.81	51.24	44.64	43.78
Nontreated	31.56	47.18	52.71	46.52	44.49
E2C+	33.91	47.04	51.37	44.10	44.11
Selected seed:					
Bayer Dust	37.59	42.42	51.82	52.96	46.20
G1	35.56	42.14	54.23	52.50	46.11
Nontreated	36.70	40.67	47.18	51.94	44.12
E2C+	35.43	40.57	54.10	49.34	44.86

All the lots of seed used in these experiments were studied by germinator tests to determine the amount of disease present. It was found that all lots were unusually free from infection and could be considered comparable to what was designated in previously reported experiments as nearly-disease-free.

The object of experiments with this type of good seed was to determine the noninjurious nature of the disinfectants used. Increases in yield following treatment of this kind of seed are not expected unless plantings are closely followed by adverse growing conditions.

The results of these experiments show that G1 does not injure good seed and may increase the yields of good seed as much as two bushels per acre.

SUMMARY

Organic compounds of mercury having the organic radical derived from furfural have been discovered especially applicable as fungicidal dusts for the control of the dry-rot seedling blights of corn. These are: (1) The compound formed when mercuric chloride and hydrofuramide are brought together in solution; (2) the compounds formed by adding mercuric chloride and mercuric nitrate to furfural after it has undergone the Cannizzaro reaction; and (3) mercury furacrylate.

The field studies on the effect of these fungicidal dusts involved obtaining yields from approximately 1,000 plots of not less than 30 hills, each, near Ames, Iowa; from about 500 plots in Illinois; and from 320 plots in four counties of Iowa.

A number of varieties, strains, and crosses of corn, as well as lots predominantly infected with each of the dry-rot organisms, were used in investigations.

The field stands and acre yields from Diplodia-infected seed were always increased appreciably by the better treatments, sometimes amounting approximately to 50 per cent. Basisporium-infected seed responded by increases in stand and yield of approximately 10 to 20 per cent and Gibberella-infected seed, five to 10 per cent. The effect on nearly-disease-free seed was not significant, but the good seed responded in each instance by increases of about two bushels per acre in the State yield contests in four districts.

The most promising of these furfural-mercury-dust disinfectants were given further study in 1928.

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A RICKETTSIA-LIKE MICROORGANISM IN EUTETTIX TENELLUS (BAKER), THE CARRIER OF CURLY TOP OF SUGAR BEETS

OLIVE SWEZY AND HENRY H. P. SEVERIN[1]

INTRODUCTION

The presence of Rickettsia or Rickettsia-like microorganisms has been recorded from a wide variety of insects within recent years, both as intestinal and as intracellular parasites. Some of these have been connected with various diseases, though the exact relations between them are still obscure in most cases. The majority of them, however, thus far bear no such ill repute. In view of the constantly-increasing importance of insects as carriers of disease, especially in plant pathology, any microorganisms which they harbor may well be made the subject of careful investigation. The presence of such bodies in the beet leaf hopper, *Eutettix tenellus* (Baker), the carrier of the curly-top disease in sugar beets, thus presents a problem which cannot be overlooked in a search for the causal agent of this disease.

Early investigations on the causal agent of curly top were confined mainly to studies of the plant; hence, when the present work was begun, attention was largely given to conditions in the insect which carries this disease. Earlier investigators found no organism associated with curly top in the plant and it has been the consensus of opinion that none exists. Smith and Boncquet (7) isolated and described a bacillus from the lesions produced by curly top but found the same organism in healthy sugar beets and concluded that it had no causal connection with the disease.

Earlier students of the problem were hampered by an inability to produce the disease by inoculation in a sufficiently large number of cases to make experiments practicable. This difficulty was overcome by methods perfected by Carter (2, 3, 4) and Severin and Swezy (6), who thus opened the way for more intensive experimental work than had yet been done. The first result of this improved technique is found in the work of Severin and Swezy on the filterability of the causal agent, proving that both the infective macerated bugs and infected beet juice are capable of producing the disease when passed through fine Berkefeld filters. Coincident with this the present work was begun. Assuming the possibility of a somewhat complicated life cycle for the causal agent which might have a visible stage in the digestive canal or blood of the insect, two lines of attack were fol-

[1] We wish to acknowledge the assistance received from Mr. W. R. Lyon of the University Medical School in carrying on the cultures that were so important to the success of this work.

lowed. The first line consisted of a study of the infective insect, both in fresh smear preparations and in fixed and stained sections of the body and its various organs. The second consisted of cultures made from the crushed insects and beets which had been filtered.

MATERIAL AND METHODS

The methods for conducting filtration experiments on both the insect and the sugar beet were similar to those used by Severin and Swezy (6) in their earlier studies on the filterability of curly top.

For examination of the beet leaf hoppers, various methods of fixing and staining were used, including most of the standard methods of cytological, bacteriological, and protozoological work. Considerable difficulty is offered by the chitinous exoskeleton of these insects, but this was overcome in part by using those adults which had recently moulted. With these, fixation and sectioning offer no difficulties. It was not always expedient, however, to confine the work to these individuals, though they gave the most successful results, and attempts were made to section the insect without removing the chitin. This, of course, resulted in the loss of much material but a sufficient number of good sections were secured to give the required data.

In addition, the intestinal canal and salivary glands also were dissected out, fixed, sectioned, and stained. Smear preparations were made of these organs and of the blood. A fairly satisfactory method of examining the stomach was found in removing the intestinal tract and flattening it between a slide and cover slip or two slides, with considerable pressure, being careful to avoid crushing the organs. If these are allowed to dry slightly before being placed in the fixing fluids the preparation will adhere to the slide and may be stained by the usual methods.

In making cultures of both the leaf hopper and the sugar beet the same methods were used as described for earlier filtration experiments (Severin and Swezy (6)). The leaf hoppers were macerated in a fluid consisting of one-half steam-extracted beet-root juice and one-half five per cent beet sugar. Enough fluid was added to make 100 cc. for filtering. After this material was passed through a fine Berkefeld (W) filter, cultures were made by using 1 loop of the filtrate and 16 cc. of the prepared culture medium and the test tubes sealed with a preparation of equal parts of paraffine and vaseline. Several media were tried and some cultures were made by using steam-extracted root juice alone. The most successful medium was a preparation containing hypophysis. This was made as follows:

Egg white		Locke's solution	.9 per cent
Hypophysis		NaCl	8.5 per cent
Dextrose	.5 gr.	$NaHCO_3$	.05 gr.

Magnesium chloride			.0005 gr.
H_2O	1 liter	KCl	.042 gr.

Subcultures were made at varying intervals, using 1 loop of the original culture to 16 cc. of the sterile culture medium.

When sugar beets were used they were ground in a meat grinder, and the juice was strained out through cheesecloth and filtered. Cultures were made of the filtrate in the same manner, using the same proportions as with the leaf hopper. In all cases the cultures were studied by making smears of the contents, fixing immediately in Regaud's or Schaudinn's fixing fluids, and staining with Giemsa. Smears also were made of the filtrate immediately after filtering and were treated in the same manner. In preparing these cultures both the noninfective and infective insects were used and both healthy and diseased beets.

MICROORGANISMS IN THE LEAF HOPPER

The intestinal canal of the leaf hopper often contains microorganisms in enormous numbers. These are found in greatest abundance in the lumen of the midintestine, though occasionally a few can be found in the oesophagus anterior to the oesophageal valve (Swezy, 8). When only a few are present they are more frequently found in the narrow portion of the midintestine than in the enlarged portion.

They also occur as intracellular parasites. Occasionally a cell in the wall of the stomach will be found greatly distended and its interior completely filled by a mass of bacilli (Fig. 1, A). In other cases a few only of the microorganisms are present in a single cell. These infected cells are most frequently those in the wall of the midintestine though infected cells are found in other parts of the body, as the fat cells in the dorsal and posterior regions and the cells in the posterior portion of the oesophagus near the oesophageal valve (Fig. 1, B). These organisms are also found in the blood. They are rarely found in the Malpighian tubules. The salivary glands have also been free of infection.

As intracellular parasites they seem to be restricted to the cytoplasm. The only exceptions to this that have been noted have been the hypertrophied cells of the midintestine where all available space in both nucleus and cytoplasm seemed to be occupied.

These microorganisms are present in the intestine of both the infective and the noninfective insect, though the former shows a higher percentage of infection than does the latter group. In 333 noninfective insects 41.4 per cent showed the presence of these organisms, while 475 infective insects showed 69.5 per cent infection. These examinations were of the intestinal tract alone. When sections of the entire body are examined the percentage of infection in the infective insect is somewhat higher, reaching about 84 per cent.

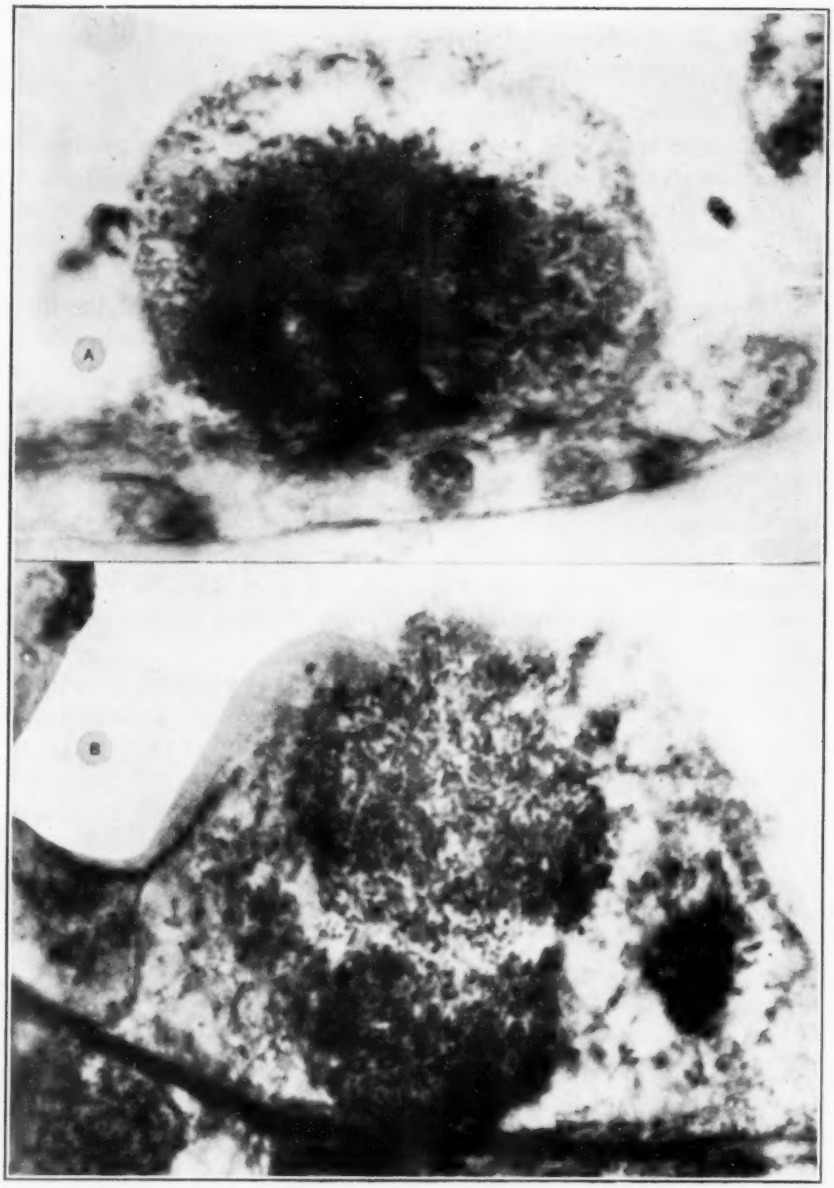

FIG. 1. A. Hypertrophied cell filled with bacilli in the epithelium of the midintestine of *Eutettix tenellus*. B. Hypertrophied cell filled with bacilli in the epithelium of the oesophagus immediately anterior to the oesophageal valve.

In appearance these organisms are diplobacillary in form with ends somewhat rounded, and ranging in size from 0.24 to 3 microns in length. In both staining capacity and sharpness of outline considerable variation is found. In many insects large numbers are present which stain clearly with iron haematoxylin, while others seem to have taken no stain. The same variations may be noted when Giesma is used. An insect is often found with the midintestine filled with a pale grey mass which presents no visible staining reactions, and only the most careful study reveals the fact that these masses are made up of diplobacillary organisms. In this type of organism the outlines, even when stained, are less clear than those of the first type, though they are rather more distinct than in the Rickettsia that have been described from other insects. Both types are found in infested cells but rarely in the same cell.

CULTURES FROM THE LEAF HOPPER

The cultures made from the leaf hopper may be divided into three groups. In the first the entire insect was macerated; in the second the intestinal canal was dissected out and ground up; and in the third the salivary glands alone were used. Large numbers of insects were used in the first group, sixteen hundred to two thousand, making approximately sixteen insects to one cubic centimeter of liquid to be filtered. In the second and third groups dissections were made of from fifty to one hundred insects for each experiment. In the first group ten filtrations were made, beginning on March 19 and ending on July 31. In the second group three filtrations were made and in the third group ten filtrations during the same interval of time. In addition to these, two filtrations were made of macerated leaf hoppers to determine the thermal death point and cultures made from these were examined in the same manner, making a total of twenty-five filtration experiments on the beet leaf hopper.

In most of these experiments smears were made of the filtrate immediately after filtration. These were found to be negative except in one instance where the slides were left exposed to the air for considerable time in drying, and a few sporebearing bacilli were found in the preparation. Cultures were made of the filtered culture medium alone, and slides prepared from these also gave negative results.

Two of the cultures made with salivary glands were examined at the end of twenty-four hours. In the other cases the first examinations were made at the end of two days or later, in some instances. Microorganisms were found in the cultures from each of the twenty-three filtrations as well as the subcultures made from them. Organisms were found in the cultures which had been heated to 65° C. but none in those which had been treated with higher temperatures.

Four attempts were made to secure cultures from the excrement of infective beet leaf hoppers. About three thousand were used each time, five hundred being placed in each of six sterilized test tubes for periods ranging from fifteen to thirty minutes. At the end of this time they were removed and steam-extracted root juice was added to each test tube. These were kept at room temperature for twenty-four hours, then filtered, and cultures made from the filtrate by the usual methods. The first three cultures were examined at the end of one, two, and three weeks and were found negative in every case. In the fourth experiment microorganisms similar to those obtained from the insect were found.

Four filtrations of noninfective beet leaf hoppers were made, using about sixteen hundred insects each time. Cultures were made of the filtrate, using the same methods and examining at the same intervals as in the case of the infective insects. These cultures gave negative results throughout.

The organisms found in the cultures from the infected insects showed a greater pleomorphism than do those in the sections of the insect. Two types predominated, one diplobacillary (Fig. 2; A, B) and the other very short, almost diplococcoid, with intergrading forms between the two. A few cultures showed a giant diplobacillary form, which was regarded as a contaminant but, later, the same organism was found in cultures which had been hermetically sealed in glass tubes immediately after filtering and heated at 70° C. for ten minutes. Their presence under these conditions suggests the possibility that they may be developmental forms of the smaller diplobacillary organism. This is only a suggestion, however.

Both the diplobacillary forms and the diplococcoid forms were found in the same cultures, in some cases in the same tubes and in other cases in tubes which had been made from the same culture but opened on different dates. In many of the cultures the diplococcoid forms appeared before the diplobacillary bodies, while in some cultures the reverse was true.

These organisms are gram-negative and, in Giemsa, show a considerable range of variation in their staining reactions. In some cultures both the diplococcus and the diplobacillary forms take a dark blue stain that is fairly sharp and clear. In other cultures both types show a lesser degree of affinity for the stain and are pale, with their borders not so well defined. They also may be stained with iron haematoxylin.

CULTURES FROM THE SUGAR BEET

The sugar beets used for preparing cultures were those showing an advanced stage of the disease. These consisted of twelve filtrations in which the prepared cultures were kept at room temperatures and two filtrations the cultures from which had been heated to temperatures varying

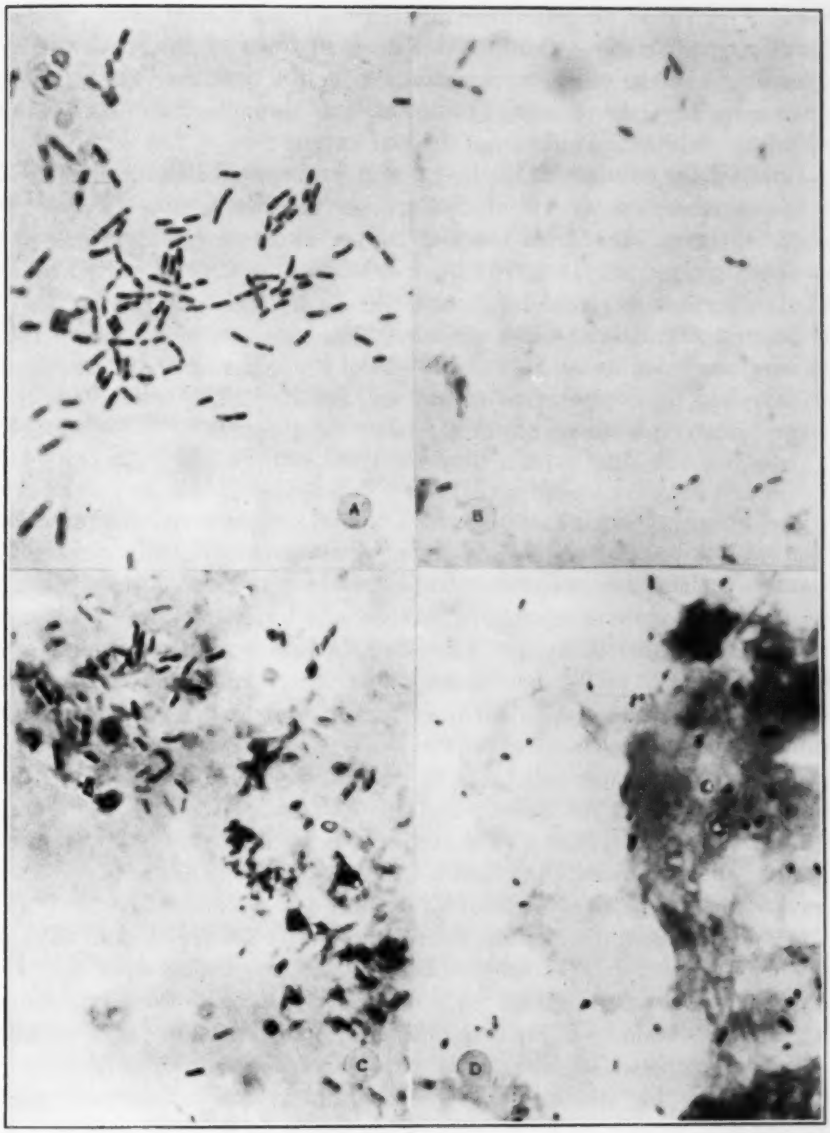

FIG. 2. A. Microorganisms from culture made from filtrate obtained by passing crushed salivary glands of *Eutettix tenellus* through a Berkefeld filter (W). B. Microorganisms from culture made from filtrate obtained by passing crushed intestinal tracts through a Berkefeld filter (W). C. Microorganisms from cultures made from beet-root juice passed through a Berkefeld filter (W). D. Diplococcoid form from culture of filtered beet-root juice.

from 65° to 75° C. to determine the thermal death point, making a total of twelve experiments. One of the first group was examined at the end of one day and the others were examined for the first time at the end of two or more days, the procedure being the same throughout as that followed in dealing with the cultures from the leaf hopper.

In all of the cultures of the first group, and also in those heated to 70° or less, microorganisms were found morphologically similar to those obtained in the cultures from the leaf hopper and showing the same pleomorphism (Fig. 2; C, D). The number of these organisms varied in different cultures, being abundant in some and very scanty in others, though the amount of material used was about the same in each culture tube. The same variation in the appearance of the two types of organisms, diplobacillary and the diplococcoid forms, was found in these cultures with a slightly greater predominance of the latter forms.

DISCUSSION

The group of organisms described under the name of Rickettsia or rickettsia-like bodies has come to include a large number of bacteria-like organisms of still somewhat doubtful classification, which are widely distributed in insects and arachnids. As defined by Arkwright, Atkin, and Bacot (1921), the Rickettsia are characterized by their minute size, usually being 0.2 to 0.5 micron in diameter; their pleomorphism from coccus-like and diplococci to bacillary forms; their resistance to ordinary aniline stains; their loss of gram stain and affinity for Giemsa; their absence of motility; their resistance to cultivation on ordinary media; and their occurrence in the intestine of blood-sucking insects. Hertig and Wolbach (5) further suggested that the term be limited to the organisms of proved pathogenicity. As pointed out by Ward (9), the latter characteristic, as well as size, is of doubtful value for classification.

The position of the organisms found in *Eutettix tenellus* and in the cultures made from it and the infected beet is open to some question, and, for the present, no attempt will be made definitely to allocate these organisms. Their affinities seem to be with the bacteria, however. They possess some of the characteristics of Rickettsia but differ from them mainly in their greater affinity for stains and in their motility. Regarding their pathogenicity nothing can yet be said either for or against their connection with the disease of sugar beets. That the same type of organism may be secured by filtration-culture experiments with the infected beet and infective insect is suggestive of a causal relation between them, however. The rather wide range of variation in size of the organisms present in the cultures is extended when the form small enough to pass the filters is included. That this latter type is an ultramicroscopic one is indicated by two facts: (1)

No organisms have been found immediately after filtering; and (2) none have been found in the numerous sections of salivary glands, though filtered cultures of the glands will give the same forms present in cultures from the entire insect. In making the cultures from the salivary glands the insects are placed in a small vial partly filled with sterile physiological salt solution. The vial is shaken before removing the insects to partially immobilize them, after which they are placed in fresh solution in a flat watch glass under the microscope for dissection. The glands are removed while the insect is immersed in the fluid and removed to a small stender dish containing sterile physiological salt solution. When this is done with care the intestinal tract and other organs of the body are left intact, only the head being removed. With the amount of salt solution used the possibility of contamination is reduced to a minimum, though there is always the chance that a few organisms from the blood may be carried over when the salivary glands are removed from the watch glass. Since the diplobacillary forms are usually present only in the intestinal tract and when found elsewhere are very scanty in numbers, the possibility of the cultures becoming infected in this manner is remote.

The form present in the sugar beet is also an ultramicroscopic one, since no visible organisms have been found in the diseased plant tissues, with one exception; sometimes the cells in the periderm of the beet root show from few to many bacilli similar to those in the cultures.

SUMMARY

It is evident that *Eutettix tenellus* harbors two different organisms, though these cannot be separated on morphological grounds. The differences between these two bacilli lie in the fact that one may be filtered and the other cannot pass through the fine Berkefeld filters; the one being found in infective insects and the other being present in both. This difference is evidently one that will be found closely correlated with differences in the life histories when these shall have been worked out more definitely.

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FURTHER NOTES ON *BACTERIUM TUMEFACIENS* AND ITS HOST RELATIONSHIP[1]

J. Ben Hill, Wm. H. Brittingham, Frances P. Gibbons, and Grace W. Watts

It has been shown by one of the authors (2) that *Bacterium tumefaciens* Smith and Town., in common with several other species of bacteria, migrates through the host tissues in the form of zoögloeae. In the present report the writers wish to assemble several additional facts bearing on the migration of this organism.

To obtain the tobacco stems used in these investigations old plants of the flowering tobacco, *Nicotiana affinis* Moore, were cut back to induce new succulent growth and these new, vigorously-growing shoots, 0.3 to 0.5 cm. in diameter, were inoculated in the internodes 0.5 to 1 cm. from the apex. The pieces of stem containing the inoculum were fixed for sectioning in alcohol-acetic acid-formalin solution, at intervals of 5, 10, 15, 20, 30, and 45 minutes, and 1 hour. This material was imbedded in paraffin and, after cutting, the sections were stained with Flemming's triple stain. Of the strains of *Bacterium tumefaciens* used in these studies "peach," "willow," "euonymus," and "pecan" were originally obtained from the laboratory of Dr. Erwin F. Smith, Bureau of Plant Industry, Washington, D. C., and "Wisc. 2004," "Wisc. 2141," and "Wisc. 2018" from Professor Wm. F. Banfield, University of Wisconsin, Madison, Wisconsin.

MIGRATION OF BACTERIUM TUMEFACIENS IN THE XYLEM VESSELS OF THE HOST[2]

Robinson and Walkden (4) and Hill (2) show that *Bacterium tumefaciens* migrates through the xylem vessels of the host. Hill indicates that the bacteria migrate vertically both upward and downward on gaining entrance to the lumen of the vessel after the wall is broken. It is the purpose of this note to report investigations conducted to ascertain the manner, direction, and rate of migration of *Bact. tumefaciens* in the xylem vessels of tobacco.

Since *Bacterium tumefaciens* is dependent upon the rupture of the walls of a cell to gain entrance to the lumen, a special inoculation technique was

[1] Contribution from the Department of Botany, The Pennsylvania State College, No. 70. Published with the approval of the Director of the Agricultural Experiment Station as Technical Paper No. 478.

[2] Investigation by Wm. H. Brittingham.

developed for the study of migration through the vessels. A scalpel was ground until it was narrow, flat, two-edged, and tapering to a point. By inserting this instrument into the shoot at right angles to the stem axis, it was possible to cut several vascular bundles and bring bacteria into contact with many severed xylem vessels. An effort was made to introduce a maximum dosage of bacteria in each inoculation. By regularly cutting pieces of the stem so that the puncture was nearer one end than the other, the part above the point of inoculation could be distinguished from the portion below.

In every section studied numerous zoögloeae were found in the xylem vessels both above and below the puncture. The tips of these zoögloeae revealed in longitudinal sections of the stem were both rounded and irregular in outline. In those of irregular outline there were frequently several lobes or advancing points. This type of zoögloeal tip is characteristic of zoögloeae occupying relatively large spaces, as has been shown by Haber (1) and Hill (3).

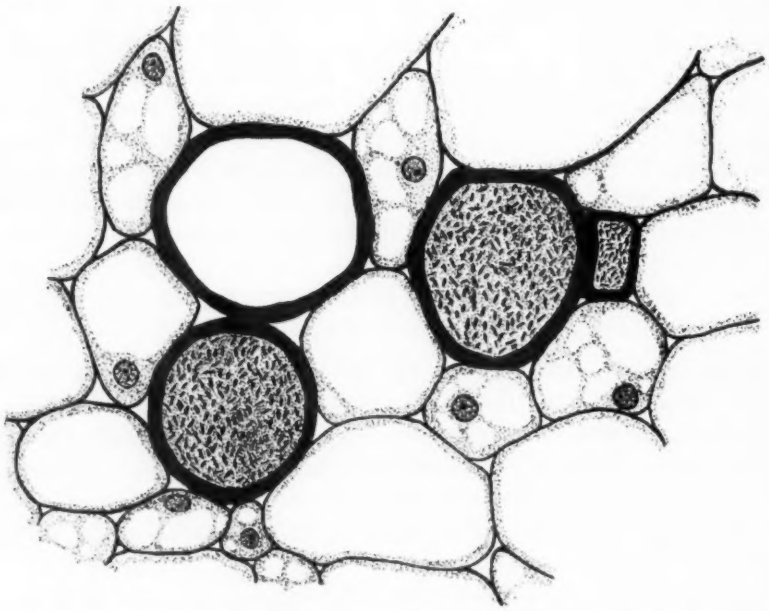

FIG. 1. Transverse section of stem of tobacco showing vessels whose lumina are occupied by zöogloeae of *Bacterium tumefaciens*.

The rates of migration in the vessels were determined from the study of the transverse sections (Fig. 1). Since all of the sections were each 12 microns in thickness, it was easy to calculate the maximum distance from

the puncture which the zoögloeae advanced in the period preceding fixation. It was found that within 15 minutes the zoögloeae advanced 2.378 mm. through the xylem vessels above the puncture and 2.161 mm. through the vessels below the puncture.

COMPARISON OF BEHAVIOR OF BACTERIUM TUMEFACIENS IN TISSUES OF VARIOUS PLANT HOSTS[3]

In connection with a study (2) of *Bacterium tumefaciens* in the tissues of tomato, *Lycopersicum esculentum* Mill., various other plant hosts, such as tobacco and castor bean, *Ricinus communis* L., were used as checks in the investigation. These have provided material for interesting comparisons of the behavior of the parasitic organism in the various hosts.

The zoögloeae of the parasite correspond in size and shape to the intercellular spaces they occupy. Thus in the intercellular spaces of the pith of the tobacco, which are much larger than those of the tomato, the zoögloeae are much larger than those in the corresponding position in the tomato; the advancing ends of the zoögloeae often are irregular, with several lobes or advancing points, while those in the narrow intercellular spaces of the tomato have rounded, blunt ends. Such irregular zoögloeal tips resemble those described above as occurring in the vessels of the tobacco.

The anatomical structure of the host plant probably determines the path of migration of *Bacterium tumefaciens* through the tissues. This appears to be particularly in evidence where there is considerable diversity in the structure of the various hosts. In the stem of the tomato plant there is a subepidermal layer of loose parenchyma tissue two or more cells in thickness, while the tobacco and castor bean have no such layers. In a former paper (2) it was shown that, following inoculation, the subepidermal layer in the tomato plant afforded a path of migration for the parasite. No zoögloeae were observed in the cortex proper of the tomato, although the pith tissue was regularly invaded by the zoögloeae, advancing into its intercellular spaces. In contrast with the situation in the tomato, the tobacco stem appeared to be invaded only by way of the intercellular tissues of the pith. A study of the migration of *Bact. tumefaciens* in the tissue of the castor bean, *Ricinus communis*, shows that the intercelluar spaces of the pith are regularly penetrated by the zoögloeae and that, in addition, zoögloeae penetrate the cortical tissue by way of the intercellular spaces but apparently not the subepidermal layer. The larger size of the intercellular spaces of the cortex of the castor bean, as compared with those of the tomato, is probably related to the use of this tissue as a path of migration in the former and not in the latter host. Following inoculation, the

[3] Investigation by J. Ben Hill.

galls produced on these hosts in a given period are largest on the tomato and smaller on the castor bean and tobacco. The probability of a correlation between the location of the path of migration and the size of galls formed by *Bact. tumefaciens* in its various hosts is suggested by the observed variation in the relative sizes of galls produced on the different host plants as well as by the facts here presented in connection with the anatomical structure and the path of migration.

COMPARISON OF THE MOTILITY OF BACTERIUM TUMEFACIENS IN CULTURE AND IN THE HOST TISSUE[4]

Another of the aspects of *Bacterium tumefaciens* which has been studied is its motility. The question arose as to whether the organism was, as E. F. Smith (5) says, "a small, white, motile, polar flagellate, rod-shaped schizomycete" and whether this could be said of the organism both in artificial culture media and in the zoögloeae in the tissues.[5] The technique of flagella staining was so uncertain that the hanging drop was used as an index of motility. Young tomato stems and beef broth were inoculated with a culture of the "peach" strain of *Bact. tumefaciens.*

At the end of 18 hours bacteria in the hanging drop from the broth showed motility, but the plant juice, squeezed from the surface of the stem cut at the point of inoculation, showed many bacteria, *all nonmotile.* These inoculations in broth and tissue were made simultaneously, and the drops observed in close succession. Over a period of 10 days, twenty-five to thirty observations all confirmed these findings. To make certain that the bacteria-like, nonmotile bodies seen in the plant juice in the hanging drop were bacteria, a plate dilution of a drop of the juice was made, and isolations into beef broth became motile at the end of 18 hours. These same bacteria had been nonmotile in the plant tissue 18 hours before. Also to show the presence of bacteria in the hanging drop of plant juice which showed no motile bodies, twelve tomato plants were inoculated directly with the expressed tomato juice, and galls were formed in every case.

COMPARATIVE STUDY OF THE BEHAVIOR OF CERTAIN STRAINS OF BACTERIUM TUMEFACIENS IN PLANT TISSUE[6]

Investigations of various workers, particularly Smith (5), have shown the existence of a large number of strains of *Bacterium tumefaciens* which

[4] Investigation by Frances P. Gibbons.

[5] This study was the result of a suggestion by Dr. H. R. Rosen, of the University of Arkansas, who, in conversation with the senior writer, expressed the thought that certain parasitic bacteria may be nonmotile in plant tissue.

[6] From a thesis submitted by Grace Weston Watts in partial fulfillment of the requirements for the degree of Master of Science at The Pennsylvania State College, published with the approval of the Dean of the Graduate School.

show a considerable range in virulence. Recent work by Hill (2) in calculating the rate of growth of the zoögloeae of *Bact. tumefaciens* has furnished a new method for a comparison of the strains of this organism.

This investigation was undertaken to secure a comparison of certain strains of *Bacterium tumefaciens* and to determine whether any correlation exists between the virulence of these strains as expressed by gall formation and development and the rate of migration of the bacteria through the tissues. With preliminary work on the virulence of strains as expressed by the rapidity of gall formation as a basis, certain strains, showing a wide range of virulence, were selected for the investigation.

For the preliminary studies, seedling tomato plants about 20 cm. in height were inoculated in the internodes between the cotyledons and the first leaves. Two inoculation punctures intersecting at right angles were made in each plant. Several plants were inoculated with each of the strains and observed daily for several weeks. After some information had been gained concerning the relative virulence of the strains as expressed by the rapidity of gall formation, three strains, peach, willow, and Wisc. 2004 were selected for intensive study. These strains represented the extremes of the range of virulence. Of these, the peach strain gave macroscopic evidence of its virulence by gall formation in from three days, at the earliest, to five days, at the latest. The galls produced by Wisc. 2004 and the willow strains were slower in their development and never attained

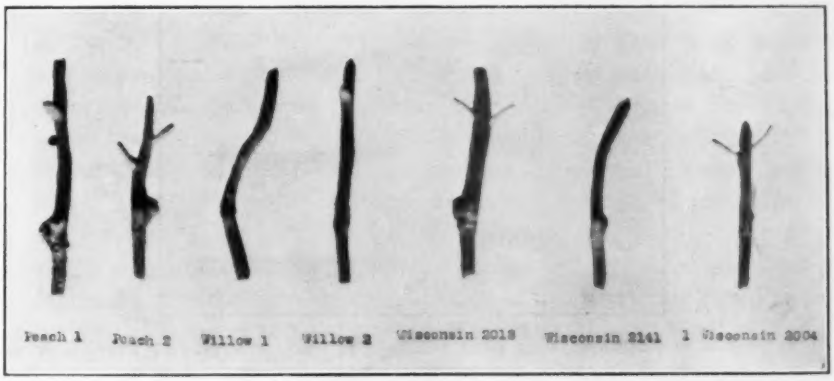

FIG. 2. Stems of tomato showing galls produced by various strains of *Bacterium tumefaciens*. Inoculation June 30, 1927. Photograph July 25, 1927.

the size of those produced by the peach strain. These two latter strains were hardly distinguishable on the basis of the rate of their gall production, both the Wisc. 2004 and the willow producing recognizable galls only after a period of five to twelve days. The largest galls were produced

on plants inoculated with the peach strain and the smallest on those inoculated with the Wisc. 2004 (Figs. 2 and 3).[7]

Since the tobacco plant provides better material for sectioning than does the tomato, the former was selected as a host in the study of the rates of migration of the strains selected for intensive study. For this phase of

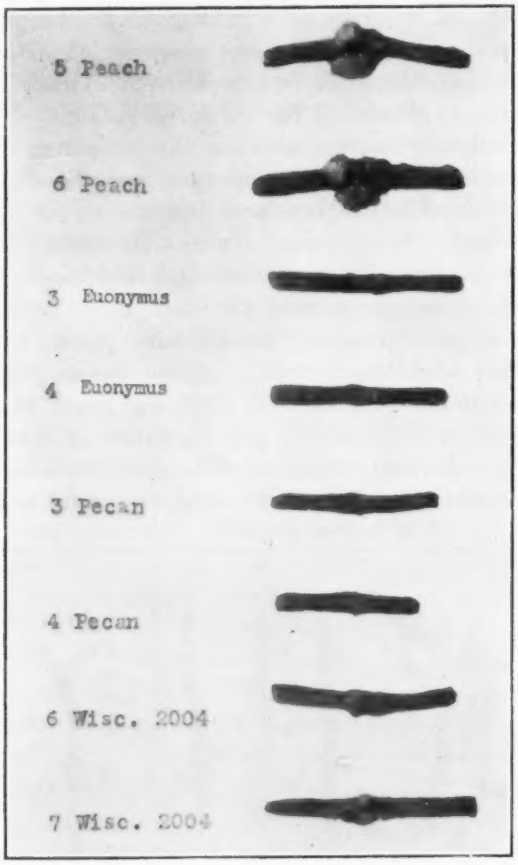

FIG. 3. Stems of tomato showing galls produced by various strains of *Bacterium tumefaciens*. Inoculation July 26, 1927. Photograph August 25, 1927.

the study, inoculation with the peach, willow, and Wisc. 2004 strains was accomplished by making a single stab with the needle. Microscopic study of these sections of the tobacco stems showed the zoögloeae located in the intercellular spaces of the pith. They were growing in most instances in

[7] Acknowledgments are due Mr. C. W. Smith, College Photographer, for assistance in photography.

a vertical direction from the puncture. Zoögloeae were more abundant in the peach and willow than in the Wisc. 2004 strain, although there seemed to be no difference in the size of the individual zoögloea of the different strains.

When zoögloeae were located, measurements were made from the point of puncture to the remotest part of the zoögloeae. The rates of migration calculated (Table 1) are the result of the study not of one or two stems

TABLE 1.—*The comparative rates of movement of different strains of Bacterium tumefaciens in tobacco stems*

B. tumefaciens	Average distance penetrated in 15 min.	Average distance penetrated in 30 min.	Average distance penetrated in 45 min.
"Peach" strain	.7668 mm.	2.13325 mm.	2.2288 mm.
"Wisc. 2004" strain	.5068 mm.	.987 mm.	1.1593 mm.
"Willow" strain	.5355 mm.	.8194 mm.	.79625 mm.

but of many sections from many stems representing several separate inoculations. For example, the rate given in table 1 for the peach strain is an average of forty-nine separate measurements from ten or more inoculations. When zoögloeae were found in identical positions on three successive sections, they were assumed to be the same zoögloea, and only one measurement was made, namely, that of the part that ended farthest from the puncture. Zoögloeae in the broken tissue at the edge of the puncture were not measured unless their remotest ends extended beyond the tissues disorganized by the puncture. Zoögloeae of peach, willow, and Wisc. 2004 were found at distances from the point of puncture, varying from a fraction of a millimeter to several millimeters.

In comparing the results of the studies on migration and those on the virulence of different strains as evidenced by the rapidity and size of gall formation, a direct correlation appears between the virulence of each strain and its rate of migration. In peach, for instance, a large gall is formed during a period of twenty to thirty days, and in Wisc. 2004 the gall formed during the same period is almost negligible (Figs. 2 and 3). An inspection of the results of the studies on migration as indicated by table 1 shows differential rates of migration for the three strains under consideration. These rates of migration are, in general, in direct correlation with the virulence of the strain as expressed by gall formation. The peach strain, which produces larger galls and forms them more rapidly than the

other strains, has a more rapid rate of migration than Wisc. 2004 and willow. This was true every time measurements were taken.

Work done by Hill (2) shows that there is a decided decrease in the rapidity of the rate of migration of the bacteria as they continue their development in the host tissue. A similar decrease in the rate of migration is also found in this investigation. There is an interesting comparison between the rate of migration of the peach strain of *Bacterium tumefaciens* in tomato tissue as worked out by Hill (2), namely, 0.04 mm. per minute, and that of the same organism in tobacco tissue in this investigation, that is, 0.05 mm. per minute, in both instances computed from sections fixed 15 minutes after inoculation.

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THE ZOÖGLOEAE OF *BACTERIUM TABACUM* AND THEIR RELATION TO THE PROBLEM OF THE MIGRATION OF BACTERIAL PHYTOPATHOGENES THROUGH THE HOST TISSUES[1]

J. Ben Hill[2]

INTRODUCTION

Within recent years several contributions have been made relative to the topic of the migration of bacterial phytopathogenes through the tissues of the host. Nixon (12, 13) and Haber (8) showed that *Bacillus amylovorus* Burr. migrates in the form of zoögloeae through the tissues of the stem and leaf of the apple. Beach (4) described the zoögloeae of *Bacterium vignae* Gardn. and Kendr., parasitic in the tissues of the leaves and pods of the lima bean. Hill (9, 10) reported the presence of zoögloeae in *Bact. tumefaciens* Smith and Town. and the rate of migration in the tissues of the stem of the tomato. All of these authors have emphasized the importance of zoögloeae in relation to the migration of these organisms.

Arthur (1), in 1886, in a study of the biology of *Bacillus amylovorus,* considered the zoögloeae observed in liquid culture to be the most characteristic feature in the life history of that organism. Bachman (3), investigating the migration of *B. amylovorus,* said "the bacteria are surrounded by a film of liquid which it seems likely has been drawn from the cell sap."

Robinson and Walkden (19) and also Riker (16, 17, 18) considered the migration of *Bacterium tumefaciens.* The former authors were primarily interested in other phases of the behavior of the organisms and the latter apparently had a misconception of the actual method of migration of the parasite.

When the earlier investigators, Atkinson (2), Beyerinck (5), Dawson (6), Frank (7), Kny (11), Peirce (14), Prazmowski (15), Tschirch (21), and Ward (22) focused their attention on the nodule-forming bacteria of the legumes, among other questions they considered was that of the migration of the causal organism. During the period between 1879, when

[1] Contribution from the Department of Botany, The Pennsylvania State College, No. 69. Published with the approval of the Director of the Agricultural Experiment Station as Technical Paper No. 477.

[2] I am indebted to Mrs. Helen D. Hill and to my colleagues in the Department of Botany at The Pennsylvania State College for criticism of this manuscript. Credit is also due the College Photographer, Mr. Chas. W. Smith, for assistance in photography.

Frank (7) discussed the nodule-forming organism of the Leguminosae, *Bacterium leguminosarum* Frank (*Rhizobium radicicola* Hiltner and Storner) (*Schizia leguminosarum* Frank) (*Bacillus radicicola* Beyerinck) (*Pseudomonas radicicola* Moore), and 1902, when Peirce (14) described the migration of that organism in the bur clover, several investigators described the manner in which these bacteria pass through the root and nodule tissues of various legumes. Notwithstanding the confusion surrounding the whole question of nodules on the roots of legumes and the various opinions as to the nature and even the identity of the causal organism and the diversity of terms used in describing its advance through the tissues, there was considerable agreement among these early authors as to the actual path and method of migration of the causal organism. All agreed that it occupied a position within the lumina of the cells. The advance through the tissues was accomplished by some kind of filamentous mass or structure to which were applied different names, such as bacterial filaments, bacterial tubes, plasmodia, infection threads, infection hyphae, infection tubes, and zoögloeae. Smith (20) gave a fairly comprehensive bibliography on nodule-forming bacteria of the legumes but with very brief abstracts of the papers and with little attempt to correlate their ideas on migration. In the preparation of this manuscript all of the articles cited have been consulted. Though these early investgators entertained a variety of ideas as to the nature and identity of the causal organism of the nodules, many of them both figured and described their conception of the method of migration of the organism. In several instances the organism is described as surrounded by a gelatinous matrix in the form of branching filaments which penetrate the cell walls and pass from cell to cell.

In the first studies on wild fire of tobacco, Wolf and Foster (23) reported that in the early stages of the disease the causal organism *Bacterium tabacum* Wolf and Foster occupies the intercellular spaces of the leaf of the host. In presentation of this report of the study of the zoögloeae and migration of the causal organism of the wild fire of tobacco, *Bact. tabacum*, in the tissues of its host, the objects are: To submit evidence which shows that another parasitic bacterium, though manifesting its presence in the plant tissues by symptoms quite different from some of the forms recently studied, migrates in a manner entirely comparable with those other forms; to make comparisons of the zoögloeae of this organism with those known in other bacterial species; and to show the relation of these zoögloeal structures to the conception of bacterial migration by zoögloeae.

INVESTIGATION

Technique. The culture of *Bacterium tabacum* used in this investigation was isolated from naturally-infected tobacco seedlings grown in Lan-

caster County, Pennsylvania, and was thoroughly tested and known to produce the disease. The microscopic study was based upon material both from the original natural infections and from infections through artificial inoculations on leaves of *Nicotiana tabacum* L. Inoculation of young vigorous leaves of cultivated tobacco, growing in the open during the summer months, was made from profuse growths of *Bacterium tabacum* on agar slants. A quantity of the inoculum was smeared on the leaf and a very fine inoculating needle was pushed through this mass into and completely through the leaf. Material was fixed either in Flemming's weak solution or in alcohol-acetic acid-formalin solution at intervals up to three days from inoculation. The material imbedded in paraffin was cut about 12 microns in thickness both transversely and in a plane parallel with the epidermal surfaces of the leaf. Flemming's triple stain gave good differentiation of both the bacteria and the matrix of the zoögloeae.

The two main points in the technique for the study of the migration of any bacterial form in plant tissue are to secure the material at the proper stage in the migration and to modify the stains to secure the best differentiation of the bacteria and matrix in the tissues. With material grown in the open at summer temperatures, the period found best for showing evidence of migration of the bacteria in the wild fire of tobacco was from 48 to 72 hours after inoculation.

Observations. In the sections prepared from material fixed 24 to 36 hours after inoculation, the bacteria can be seen growing in the mass of tissue lacerated by the needle. In the material fixed 48 to 72 hours after inoculation, the bacteria may be seen in the uninjured tissue surrounding the needle puncture. Zoögloeae have been formed and these penetrate the healthy tissues, passing through the intercellular spaces and in the early stages apparently doing little, if any, harm to the cells of the host (Fig. 1, A, B). After cells have been surrounded by bacteria for an undetermined period, plasmolysis and disorganization of the protoplast are evident (Fig. 2, E). The tissue most frequently penetrated by the zoögloeae is that composed of the parenchyma cells near the smaller vascular bundles of the leaf. Though bacteria were seen in some of the smaller vessels, the invariable injury of the latter leads to the belief that the bacteria gain entrance to vascular elements only when these have been ruptured.

Microscopic study of the sections of tobacco leaves, fixed about 72 hours following their inoculation with *Bacterium tabacum*, revealed an abundance of the characteristically blunt rounded tips of the zoögloeae of the parasite arrested in place just as they pushed their way through the irregularly-shaped intercellular spaces of the mesophyll (Fig. 1, A, B; Fig. 2, C. D. E). The zoögloeae consist of bacteria surrounded by a matrix easily dif-

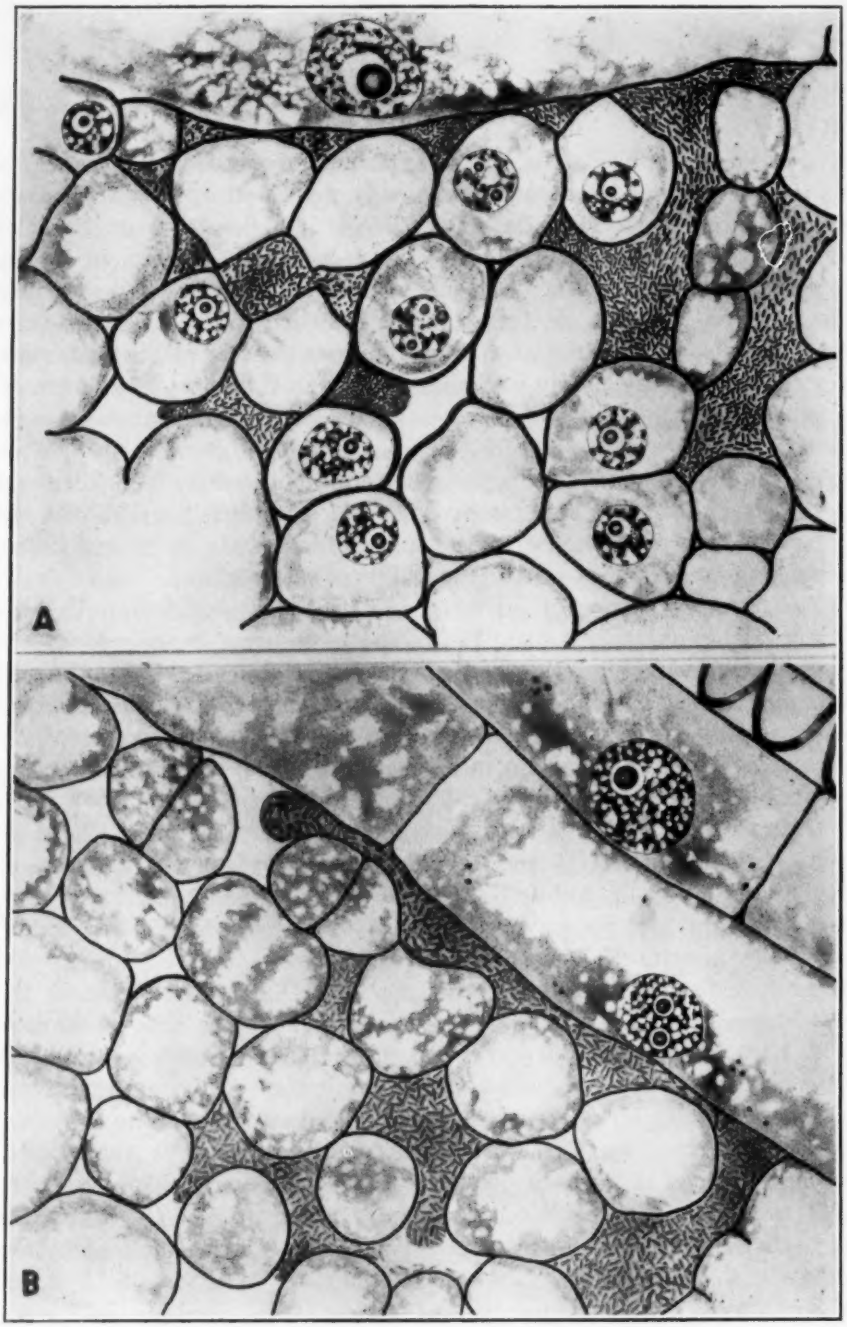

FIG. 1. A. Section of a leaf of the tobacco *Nicotiana tabacum* cut parallel with the epidermal layers, showing the distribution of the bacteria (*Bacterium tabacum*) as they invade the healthy tissues following inoculation. The region is that of the mesophyll adjacent to a small vascular bundle an elongated cell of which is shown above. At the lower left attention is directed to the two rounded tips of the zoögloeae. About 1250×.

B. Section cut through the same general region as that shown in A. One of the zoögloeae of *Bacterium tabacum* is shown advancing through the irregular intercellular spaces which lie adjacent to and parallel with a vascular bundle whose cells are shown in the upper right. The tip of this zoögloea is enlarged and rounded as it has advanced into a relatively large intercellular space. Three characteristically blunt and rounded zoögloeal tips are shown in the lower left and lower center of the figure. About 1250×.

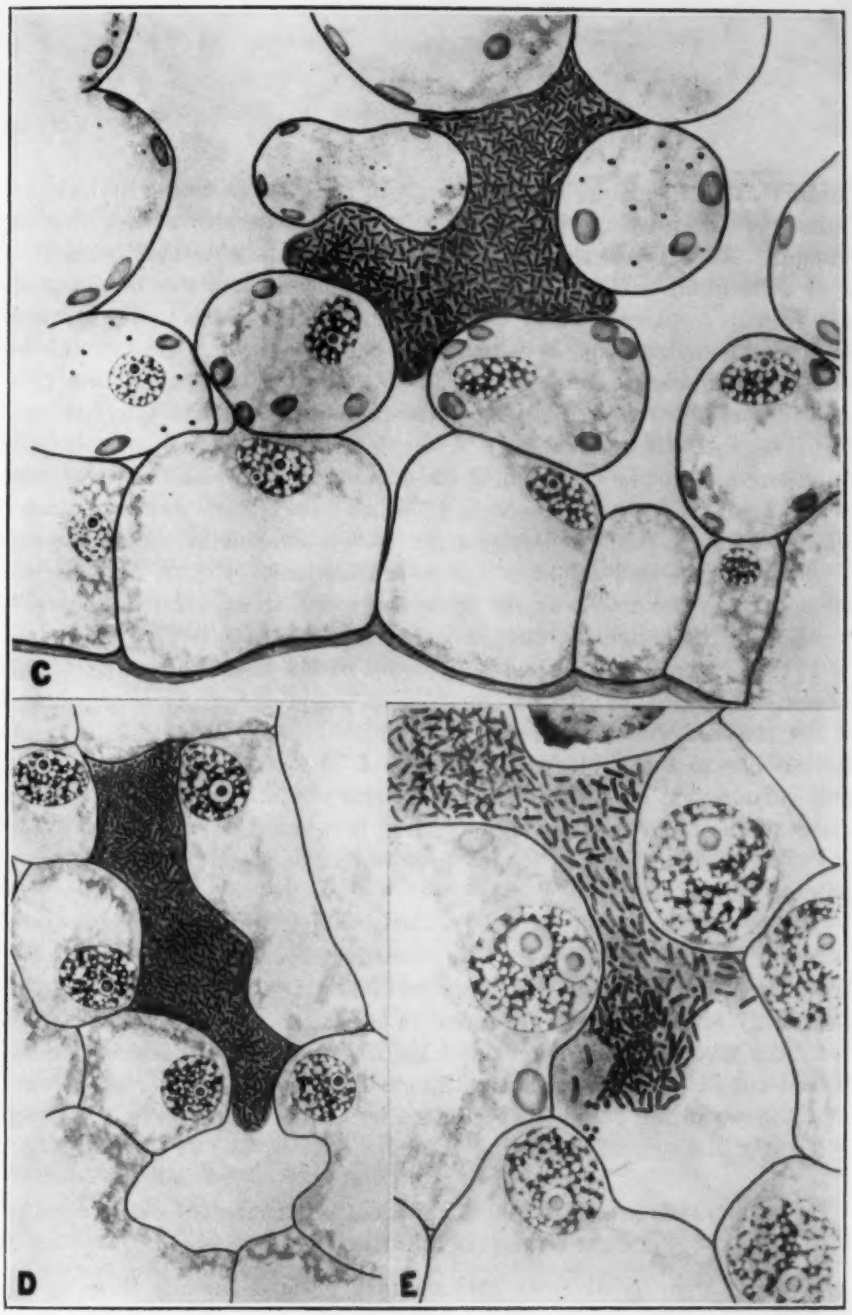

FIG. 2. Various forms of zoögloeae of *Bacterium tabacum* within the irregular intercellular spaces of the mesophyll of the leaf.

C. A transverse section of the leaf of a tobacco seedling naturally infected with "Wild Fire." The initial infection had apparently occurred on the upper surface of the leaf. The bacteria had migrated outward and downward through the intercellular spaces of the palisade layer, reaching the lower mesophyll where the zoögloea is shown. The zoögloea has assumed the irregular shape of the intercellular spaces of this region. Four blunt, rounded zoögloeal tips indicate the directions of migration of the bacteria. The cells of the mesophyll have nuclei cytoplasm and chloroplasts. The lower epidermal cells without chloroplasts are shown below. About 1250 ×.

D. A section adjacent to a vascular bundle of the leaf with an elongated zoögloea occupying a short narrow intercellular space parallel to the cells of the bundle. The rounded tip is shown about to enter a much larger intercellular opening. About 1250 ×.

E. A section through the mesophyll of the leaf showing a zoögloea advancing into a relatively large intercellular space. The zoögloea has three advancing tips. About 2500 ×.

ferentiated by Flemming's triple stain. In most instances the bacteria are stained red or purple, while the matrix shows a greater affinity for the orange G. The forms assumed by the zoögloeae indicate that the matrix is of a semifluid or jelly-like consistency and easily capable of a flowing movement. Apparently, they do not readily adhere to the walls surrounding the intercellular spaces which they occupy. In this respect the behavior of the zoögloeal mass is similar to that of a heavy fluid in a tube, the blunt rounded tips of the zoögloeae resembling the characteristic convex meniscus. Of interest also is the orientation of the bacteria with their long axes in the direction of the advancing tip which can frequently be observed where the zoögloea had pushed into a very narrow intercellular space (Fig. 1, B). Bacteria are more abundant in the older parts of the zoögloeae and fewer in the advancing tips (Fig. 2, E). In the older parts of the zoögloeae the bacteria become so crowded and stain so deeply that the matrix is often entirely masked.

Probably the most conspicuous feature of the zoögloeae of *Bacterium tabacum* in the tissues of the tobacco leaf is the presence of a large number of the rounded, blunt, advancing tips (Fig. 1, B; Fig. 2, C). A condition similar to that illustrated in figure 1, B, is very frequently found. This phenomenon is probably associated with the labyrinthine intercellular spaces of the lower mesophyll of the leaf in which the organism is found and is merely its response to its opportunity for simultaneous growth in many directions.

In the invasion of the large irregularly shaped intercellular spaces, the zoögloeae assumes the shapes of the cavities they chance to occupy (Fig. 1, B; Fig. 2, C, D, E), so that an outstanding feature of the zoögloeae is the variety of forms which are found in the lower mesophyll tissues of the leaf. An unusual form of zoögloeal tip was seen where the structure had pushed out into a relatively large intercellular space. Under such conditions the advancing face of the zoögloea became irregular (Fig. 2, E) and frequently had several advancing points.

COMPARISON OF BACTERIUM TABACUM WITH CERTAIN OTHER SPECIES OF BACTERIAL PHYTOPATHOGENES

Some very instructive comparisons may be made among those species of bacterial plant parasites the migration of which has been described. The most interesting features for comparison are the nature and form of the zoögloeae and the relative rates of migration of the bacteria through the tissues of the host. While there are many unsolved problems associated with the bacterial phytopathogenes, the features in which the published reports show greatest accord are the observations relating to the

physical properties of the zoögloeae, their responses to their environment in assuming the shapes of the intercellular spaces of the host tissues, and the relation of these facts to the general method of migration of those bacterial phytopathogenes which have been adequately investigated in regard to this feature of their behavior.

The observation of *Bacterium tabacum* in the tissues of the tobacco leaf leads to the conclusion that its behavior is similar in all essential features to that of the species of bacterial plant parasites recently described. This organism migrates in the form of zoögloeae which, at least in the early stages of the infection, are confined to the intercellular spaces of its host. The lumina of cells mechanically injured may also be occupied by the organism. The zoögloeae assume the shapes of the irregular spaces of the mesophyll and are, therefore, of so many forms that no one form may be considered as typical. The conception of the physical nature of the zoögloeae of *Bact. tabacum* based on their microscopic appearance agrees in all essential respects with that expressed by the recent workers (*loc. cit.*) concerning the zoögloeal structures in the species they studied; that is, the zoögloeae of *Bact. tabacum* consists of bacteria surrounded by an apparently gelatinous semifluid matrix and pass into the intercellular spaces of the tissues, where they assume the shapes of the cavities they occupy.

Though the study of the rates of migration of parasitic organisms is interesting, their accurate determination is beset with considerable difficulty. It must be realized that various inconstant environmental factors, such as temperature, moisture, and the condition of the host, must be considered; since these affect the rate of growth, they may also be expected to affect the rate of migration, which is probably a growth phenomenon. Differences in rates of migration may also be expected to characterize distinct strains of parasitic organisms. The rate of migration determined for one strain of an organism, therefore, may not be the rate which another strain of the same species may show. It is well known that the species of bacteria vary in many characteristics, such as the length of incubation period and the nature of the symptoms of the diseases which they produce. In the light of such knowledge, it may be expected that the various species of bacteria will show variation also in their rates of migration.

Bacterium tabacum migrates very slowly. It is extremely difficult to measure accurately the rate of migration of this parasite, since mesophyll tissue offers only a very crooked and irregular path. The fact that in this species advancing zoögloeae were found only in material fixed 48 to 72 hours after inoculation may be regarded as an indication of a slower rate of migration as compared to that of *Bact. tumefaciens* in which migration is well under way within an hour following inoculation (10).

SUMMARY

The causal organism of wild fire of tobacco, *Bacterium tabacum*, migrates in the form of zoögloeae in a manner comparable in all essentials with those species of parasitic organisms the methods of migration of which have been recently described.

The rate of migration of *Bact. tabacum* is very slow as compared with that of *Bact. tumefaciens*.

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BOOK REVIEWS

Comparative Morphology of Fungi. By Ernst Albert Gäumann. Translated and revised by Carroll William Dodge. McGraw-Hill Book Company, New York, 1928. Gäumann's "Vergleichende Morphologie der Pilze" was a noteworthy contribution to mycological literature in bringing together the advances of forty years in a field of fertile research. The announcement of an English translation was therefore most welcome.

Dodge's work is a revised translation. The sequence of the orders of the Basidiomycetes has been rearranged in accordance with Gäumann's suggestions in the original book. More or less extensive revisions have been made in the treatment of the Tuberales, the Laboulbeniales, the Polyporales, and the Gasteromycetes. Minor changes occur throughout the book. One outstanding feature of the whole work is that Dodge has taken the liberty to change a sentence, a paragraph, or a section without indicating that it is different from the original except by stating in a most general way in his preface to the American edition that he has rewritten certain sections and corrected ambiguities in the original text. In a translation, an accurate rendition of the original author's ideas is expected of the translator and it would seem best that differences of opinion or corrections be given in authenticated footnotes. As it stands the book can scarcely be called Dodge's; neither does it accurately represent the original Gäumann.

Translation of so extensive a work is a laborious and tedious task and any one undertaking it deserves much commendation. It is greatly to be regretted that more time was not taken for correction of typographical errors and for critical reading. When this sentence, "Diese Auffassungen bieten (unter der Voraussetzung, dasz die Protobasidie wirklich eine ursprüngliche Form der Basidie ist) den Vorteil, dasz sie in den Uredineen das gesuchte Verbindungsglied zwischen den Ascomyceten und den Basidiomyceten schaffen: letztere hätten sich aus ersteren über uredineenähnliche, saprophytische lebende Formen entwickelt" (page 452) is rendered thus, "These conceptions (assuming that the phragmobasidium is originally a form of basidium) offer the advantage that they create in the Uredinales the sought-for link between the Ascomycetes and the Basidiomycetes; the latter had developed from the former through the rust series to the saprophytic forms" (page 587) one loses confidence in the accuracy of the English he is reading.

Frequent mistranslation of words, typographical errors, and ambiguous sentences which lose their meaning by too literal translation and omission of essential words or phrases make an understanding of some parts of the

text very difficult. Examples of mistranslations are "flagsmut" for "Flugbrand" (page 596), "next" for "zunächst" (page 52), "shortly before" for "kurz nach" (page 81), "swelling sporangiospore" for "Sporangiospore bei schwächerer Vergröszerung" (page 96), "fuse" for "vermengen" (page 274). Typographical errors are more serious and numerous than one would expect in a work of this sort; for example, "sexual" instead of "asexual" (page 117), "conidia" instead of "coremia" (page 177), "aplanogametes" instead of "planogametes" (page 17), "zoospores" instead of "oospores" (page 62). The chapter on the Laboulbeniales, where the author has wholly abandoned the language of the original and has rewritten the text in a clear and forcible manner, stands in sharp contrast to the other chapters which are presumably free translations. There is a disconcerting lack of consistency in carrying through the whole work the author's general theme of the development of the dicaryophase in the fungi, where Dodge inserts without comment the work of certain authors not treated by Gäumann; for example, Elliott's account of the nuclear behavior in *Ceratostomella fimbriata* (page 262). The interpolations of the translator are not always accurate: for instance, attributing to Sadebeck the separation into Exoacus of the species of the Exoascaceae with 4- to 8-spore asci and into Taphrina of those in which the spores bud in the ascus (page 164).

Gäumann's work has been one of the outstanding contributions in the field of modern mycology. He has assembled a vast amount of mycological data and presented them with remarkable clarity in an orderly sequence. He has made valuable contributions on the significance of the dicaryophase as a distinguishing characteristic in the phylogeny of the fungi. If Dodge's revised translation will serve to bring this contribution more generally to the knowledge of American readers it will serve a useful purpose.—LOUISE DOSDALL, University Farm, St. Paul, Minn.

A Monographic Study of Sweet-Potato Diseases and Their Control. By L. L. Harter and J. L. Weimer. United States Department of Agriculture Technical Bulletin 99, 117 pages, 24 figures, 26 plates. Washington, D. C. 1929.—In recent years the ever-increasing tendency toward crop specialization among plant pathologists has become more and more pronounced. Coincident with and as a natural outcome of this development, the publications on a given subject have become increasingly scattered, thus working hardships upon the specialist whose library facilities are limited. The obvious solution of the difficulty is the publication of monographic studies of the diseases of individual crops by those whose personal investigational work and library facilities qualify them as specialists.

Inasmuch as phytopathology in its broadest aspects knows no political

boundaries, it is quite fitting and proper that the United States Department of Agriculture should take the initial step in the issuance of individual crop-disease monographs. In presenting to plant pathologists "A Monographic Study of Sweet-Potato Diseases and Their Control," by L. L. Harter and J. L. Weimer, the Federal Government has recognized a need and has taken such measures as fall within its province to mitigate the bibliographic conditions of less fortunate plant pathologists.

Harter and Weimer, as a result of over sixteen years of research devoted mainly to sweet-potato diseases, are admirably qualified for the responsibility of monographing the diseases of this crop, the second most important vegetable in the United States. They have brought together within the confines of 117 pages all the important available data on the diseases of sweet potatoes—now spread in the literature of over twenty countries and in more than seven languages—and have condensed and correlated certain heretofore unpublished data of their own.

The major divisions of the bulletin are made on the basis of the causal agency involved—fungi, nematodes, and physiological diseases or those of unknown cause—and their control. Each of these is further subdivided into important and minor diseases of field and storage.

Each disease is exhaustively treated in much the same general manner. The history of the malady, its geographical distribution and economic importance, the symptoms, and the causal organism are fully described. If the cause is not known, a discussion is given of the factors contributing to the presence of the disease and their possible etiological relation. Of special interest—and one of the most valuable portions of the bulletin—are the treatments of the causal organisms involved in the several diseases. These are thoroughly discussed from the standpoints of historical taxonomy, pathogenicity, morphology, life history, and dissemination.

Where a disease is of importance in both field and storage, the former phase has received the principal discussion and is only sufficiently reviewed with reference to the storage aspect to orientate the disease and its causal organism in the reader's mind.

The discussion of the control measures has been left for a major section, since the same methods or modifications of them are applicable to all. The control of the field diseases of the sweet potato is recommended through exclusion of diseased seed stock from foreign countries by means of adequate quarantine regulations, through inspection and certification of domestic seed and plants, through the use of disease-resistant varieties in so far as such may exist or may in the future be developed, and through eradication by following approved agricultural practices and seed treatment. Elimination of disease through thorough inspection of the harvested crop and the selection of disease-free stock for seed purposes, the proper methods

of digging and handling, the correct management of the storage house and the stored crop, and the fumigation—ill-advised at best—of sweet potatoes in storage are covered in the discussion of control of storage diseases.

Among the new or little-known diseases covered in the monograph, the following may be mentioned: Mottle necrosis, caused by *Pythium ultimum* and *P. sclerotcichum*; rootlet rot, caused by *P. ultimum*; a leaf spot caused by *Septoria bataticola*; a leaf spot caused by a species of Alternaria; rust, caused by *Coleosporium ipomoeae*, with an account of its parasite, *Monosporium uredinicolum*; leaf spot, caused by *Cercospora batatae*; damping off, attributed to a species of Sclerotinia similar to *S. minor*; a foot rot caused by *Macrosporium solani*; growth cracking; fasciation; intumescence; sunscald; minor storage diseases caused by *Mucor racemosus, Alternaria* sp., *Penicillium* sp., *Botrytis cinerea, Epicoccum* sp., *Gibberella saubinetii*; *Fusarium culmorum, F. acuminatum, Trichoderma köningi,* and *Sclerotinia* sp.; and minor reports on about twenty other fungi which have, in the course of years, been isolated from sweet potatoes.

The unusually clear and well-selected illustrations, of which there are fifty—24 text figures and 26 plates—,show the smallest characters of the many diseases.

A bibliography of 211 titles, including all the important known publications on sweet-potato diseases, as well as many supporting papers, is appended.

In this monographic study of the diseases of a single crop, the authors have done a commendable piece of work, enormous in scope, exhaustive in treatment, and—what is most important—simply, though none the less scientifically, written. It is to be hoped that similar monographs will be forthcoming for other crops and that, later, following the accumulation of new data, revisions will be made so that the current usefulness of the works may not be lost through becoming out of date.—W. A. WHITNEY, Washington, D. C.